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THE EFFECTS OF AN ACUTE BOUT OF STRENUOUS AEROBIC EXERCISE ON PLASMA, ERYTHROCYTE, URINARY AND DIETARY VALUES FOR SELECTED TRACE MINERALS

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

IN THE GRADUATE SCHOOL OF

TEXAS WOMAN'S UNIVERSITY

DEPARTMENT OF NUTRITION AND FOOD SCIENCES

COLLEGE OF HEALTH SCIENCES

BY

KIMBERLY K. EDGREN, RD, BS, CDE

DENTON, TEXAS

DECEMBER 1996

TEXAS WOMAN'S UNIVERSITY
THE GRADUATE SCHOOL

CERTIFICATION OF FINAL EXAMINATION

October 1, 1996
DATE OF EXAMINATON

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The undersigned have on this date examined Kimberly Gramenz Edgren for the degree of Master of Science and hereby certify that the examination has been successfully completed. This ( ) dissertation, (X) thesis, ( ) professional paper has been reviewed by each of us and is approved.

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1 OCTOBER 1996

To the Associate Vice President for Research and Dean of the Graduate School:

I am submitting herewith a thesis written by Kimberly Kae Edgren entitled "The Effects of an Acute Bout of Strenuous Aerobic Exercise on Plasma, Erythrocyte, Urinary and Dietary Values for Selected Trace Minerals." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Betty B. Alford
Betty B. Alford, Ph.D., Major Professor

We have read this thesis
and recommend its acceptance:

Cindie M. Huels

Ginger P. Schumer

Department Chair

Accepted

Associate Vice President for Research
and Dean of the Graduate School

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ABSTRACT

The Effects of an Acute Bout of Strenuous Aerobic Exercise on Plasma, Erythrocyte, Urinary and Dietary Values for Selected Trace Minerals

Kimberly K. Edgren
December 1996.

Nineteen competitive cyclists participated in a week-long study to determine if prolonged, intense aerobic exercise produced a significant change in plasma, erythrocyte, and/or urine values for zinc(Zn) and copper(Cu). Subjects pedaled a cycle ergometer for 1 hour at 80% anaerobic threshold, after which, resistance was incrementally increased by 20 watts/minute until voluntary exhaustion. Six blood collections were drawn: pre-exercise, post-exercise and 2-, 24-, 72-, and 120-hours post-exercise, respectively. Five 24-hour urine collections were taken: one day pre-exercise, the exercise day, and 1-, 2-, and 4-days post-exercise, respectively. Results were significant ($p<0.05$) for: plasma-Zn, erythrocyte-Zn, plasma-Cu, and erythrocyte-Cu changes, respectively, over the study period; post-exercise plasma-Zn increased over all other blood draws; pre-exercise erythrocyte-Zn and erythrocyte-Cu higher than each subsequent blood draw; and post-exercise plasma-Cu higher than prior or subsequent blood draws, respectively. Results suggest that Zn and Cu status cannot be maintained in plasma and erythrocytes when athletes consume self-selected unsupplemented diets.

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CHAPTER I

INTRODUCTION

Americans are increasingly aware of prevailing health recommendations to increase physical activity as a means of enhancing quality of life and minimizing risk of developing chronic disease. As participation in a variety of recreational and competitive sporting activities increases, it is important to understand the interrelationship between physical activity, nutrition and performance. In the last decade this triangular relationship, with emphasis on trace mineral nutriture, has become an increasingly researched topic. Data has shown conflicting effects on trace mineral status in athletes in response to aerobic exercise (6,20,45,71,77,118,121).

Trace minerals are critical to physical performance, particularly zinc (Zn) and copper (Cu). Zinc is a cofactor for nearly all enzyme reactions and Cu is critical for aerobic metabolism. In general, they are essential for energy metabolism, acting as inorganic regulators of various aspects of energy production and utilization, including lipolysis, glycolysis and proteolysis. Their primary biochemical functions encompass enzymatic actions, structural roles and antioxidant roles.

Many of the metabolic alterations of Zn and Cu nutriture, as a result of aerobic exercise, have yet to be established. Trace mineral status is a balance of dietary intake, metabolism, utilization and excretion. Studies are equivocal with regards to any alteration

of trace mineral status with physical training (6,22,43,71). Observations of decreased concentrations of plasma-Zn and plasma-Cu and increased excretion of Zn and Cu via urine or sweat, may indicate a loss or change in tissue distribution of these trace elements during exercise (6,14,77). Research results showing increased concentrations of erythrocyte-Zn and erythrocyte-Cu, concurrent with lower plasma-Zn and plasma-Cu levels in athletes compared to non-athletes, supposes that chronic exercise elicits redistribution of trace elements between tissue compartments (121). If physical training alters trace mineral status in athletes, then a concurrent suboptimal dietary Zn and Cu intake may abet adverse effects (6,22). Since food consumption patterns of athletes have not consistently provided recommended levels of Zn and Cu, this may lead to marginal deficiency, which in turn, could induce a direct effect on athletic performance (45,48,79,101).

Changes in Zn and Cu concentrations in plasma, erythrocytes and urine have been studied for different sports and training conditions. The variety of effects of physical exercise that have been reported may depend on the type, duration and intensity of exercise, training level of the subjects and dietary trace mineral intake (3,4,14,78,118). Research that examines the parallel effects of exercise on circulating concentrations, daily losses and dietary adequacy of trace minerals may provide evidence for increased mineral needs. This encompassing approach would gain further insight into the interrelationship between Zn and Cu, physical activity and performance (27,69-72,95,120).

Purpose of the Study

The purpose of this study was to investigate the effects of prolonged, intense, acute aerobic exercise on Zn and Cu status, encompassing the dietary requirements, fluctuations in plasma and erythrocyte trace mineral concentrations, and alteration in urine excretion of both trace metals.

Objectives of the Study

The specific objectives of the present study were to determine the following :

1. If prolonged intense aerobic exercise (> 1 hour at 80% predicted anaerobic threshhold [defined by the standard equation: 220 - age]) produces, with regard to Zn and Cu:

a) a significant decline in plasma and/or erythrocyte concentrations,

b) a shift from plasma to erythrocyte or vice versa, and/or

c) an alteration in urine excretion over a one week post-exercise time period.

2. If plasma and erythrocyte Zn and Cu status can be maintained by subjects participating in acute intense aerobic exercise while consuming a self-selected diet without supplementation.

CHAPTER II

REVIEW OF LITERATURE

The trace elements, Zn and Cu, are essential in energy metabolism. Forty percent of all people in the United States exercise or play sports regularly (131). Athletes, in particular, require adequate amounts of Zn and Cu yet, are susceptible to increased losses and suboptimal dietary intake of Zn and Cu compared to nonexercising control subjects. The biological importance of Zn and Cu are well established however, the mechanisms by which physical exercise modifies their metabolism remain unclear. Specifically, insufficient data that examines the influence of acute, sustained aerobic exercise on Zn and Cu nutriture in physically active men is available.

Zinc - Distribution in the Human Body

Zinc is present in all organs, tissues, fluids and secretions making it the most abundant trace mineral in human body tissues other than blood (27,64,86). The total body content of Zn is approximately 2.5-3.0 grams (3.8-4.5 mmol) for an adult male. Widely distributed throughout the body, Zn is most heavily concentrated in muscle (60%), bone (28%) and liver (1.8-5%) (45,64,79). Zinc is primarily an intracellular ion maintaining more than 95% of total body Zn within the cells; of cellular Zn, approximately 60-80% is in the cytosol (64).

Smaller concentrations of total body Zn are found in circulating blood. In normal human blood, erythrocyte-Zn constitutes 75-88%, plasma-Zn 12-20% and leukocyte Zn 3% of whole blood Zn (86). The majority of plasma-Zn is loosely bound to albumin (57%), but can be readily complexed to other circulating proteins (20,22,106). Zinc attached to albumin and amino acids can be delivered directly to tissues and the amino acid bound fraction determines the renal filtration of Zn (11,12,63,64,127). The total Zn in the plasma (~ 3 mg) is far less than the total amount of Zn (~ 3 g) in the major tissues; therefore, a small variation in tissue Zn content can have considerable proportional effects on plasma-Zn. The flux of metabolically active Zn through the plasma depends on a variety of factors but must be rapid to maintain a relatively constant plasma-Zn concentration.

The erythrocyte-Zn content is attributed to intracellular enzymes. Nearly 87% of total erythrocyte-Zn is a component of carbonic anhydrase isoenzymes (CA-I), while the enzyme superoxide dismutase (SOD) accounts for approximately 5-11% of E-Zn (59,94). The remaining erythrocyte-Zn is considered bound to hemoglobin, metallothionein and other metalloenzymes, such as lactate dehydrogenase (LDH) (90,94,129). Average erythrocyte-Zn and plasma-Zn values are 135-245 mmol/L and 11.5-18.5 mmol/L, respectively (106,148).

Diet and Bioavailability

Generally, plasma-Zn concentration is insensitive to changes in dietary Zn at intakes > 4 mg/day and that no correlation exists between the two (40,63,73,82). In contrast, some researchers later found that dietary Zn intake significantly correlated with erythrocyte-Zn through metallothionein sensitivity to low and high levels of dietary Zn (25,39).

The Recommended Daily Allowance (RDA) is 15 mg for adult men 19-50 years of age (86). Still, the mean Zn intake of adult self-selected mixed diets in the United States ranges from 8.6-14.0 mg Zn/day (42,51,76,101,123). Prior researchers have reported dietary Zn density from 5.0-6.0 mg/1000 kcals (6,101,104). Zinc intake has been shown to strongly correlate with energy and protein intake (82). Meeting RDA levels would seem likely considering the high energy intake of most male athletes, yet reports of dietary Zn intake are variable indicating subjects achieving (70,115), exceeding (118,119) or not meeting (26,45,88,101) the RDA.

Inappropriate food choices seem to be responsible for low dietary Zn intakes. Protein-rich foods, such as meats, seafood and poultry, contribute the greatest amounts of dietary Zn. See Table 2.1. A diet low in animal protein will have difficulty satisfying the RDA for Zn as protein enhances Zn retention (21,36). Diets with high Zn quality contain ample amounts of highly bioavailable Zn including red meats and shellfish (79,114,145). Meat provides nearly 50%, and dairy products approximately 20%, of Zn in the average

diet in the United States (33,76). The remaining 30% of dietary Zn is contributed by cereal grains which provide poor bioavailability of a smaller amount of Zn (33,64). Plant foods, notably legumes and nuts, that contain phytate and dietary fiber can inhibit Zn absorption in the gastrointestinal (GI) tract and promote Zn excretion (81,83,86,120,121). A millimolar ratio of phytate to Zn > 10 increases the risk of poor Zn absorption (68). Regardless, researchers purport that the phytate content of the average diet in the United States is not sufficiently high to impair Zn absorption (62,83). The milk protein, casein, is also considered a ligand that hinders Zn absorption (42,114).

Table 2.1 Zinc Content of Major Food Sources of *Zinc

	Zinc Content (mg per 100 gm serving)	Percent of RDA
Oysters	100	667%
Wheat germ	17	113%
Roast beef	4-8	27-53%
Cereals	6	40%
Liver	4	27%
Chicken, dark meat	3	20%
Whole wheat bread	3 (1 mg/slice)	20%
Nuts	3	20%
Legumes	1-5	7-33%
Eggs	1.5 (0.7 mg/egg)	10%
Milk	0.3 (3 mg/liter)	2%

* Modified from Dressendorfer and Sockolov 1980

Dietary Zn is involved in several nutrient interactions that affect its bioavailability. Zinc competes for absorption binding sites with other elements of similar physiochemical characteristics, such as Cu (33,64,84,142). Iron and Zn share a mutual affinity for their preferred carrier protein. This iron-zinc competition, however, should not have a major effect on Zn absorption if iron is derived from food sources under normal dietary conditions (128). High calcium intakes reportedly inhibit intestinal absorption of Zn (21,38). Certain amino acids, such as cysteine and histidine, richly found in meat, liver, eggs and seafood, enhance Zn absorbability by forming stable complexes (64).

Considering Zn content and bioavailability of select foods, higher risk of inadequate Zn intake is evident for those consuming cereal-based diets with minimal meat. Athletes are inclined to ingest diets high in carbohydrate (CHO) and dietary fiber. This composition would tend to contribute low amounts of Zn and/or provide the dietary constituents that interfere with Zn absorption (25,27,121,126). High CHO foods, such as fruit, pasta, pastry and ice cream are poor sources of Zn, yet are frequently included in an athletic diet. Endurance athletes, particularly, associate high CHO intake with enhanced performance; thus, this population may be at risk of Zn deficient intake (27,69).

Metabolism

Though nutrition surveys indicate legitimate concern for suboptimal dietary Zn intake in athletic populations, the human body appears to have compensatory mechanisms to regulate Zn uptake. When dietary Zn intake is reduced, the body adapts to stimulate Zn

absorption and decrease secretion of endogenous Zn into the GI lumen. Conversely, excessive Zn intake will have the opposite effect, thereby maintaining a relatively constant amount of Zn absorption (21,49,55,62). Generally, 20-30% of oral Zn intake is absorbed; this is variable depending on the individual and the food source (20). Zinc absorbed into the GI mucosal cell may be used for cellular Zn-dependent processes, remain intracellular Zn, or traverse the cell and enter the portal blood bound to albumin. Circulating Zn is redistributed to other Zn-demanding tissues, principally muscle and bone.

Zinc has three primary routes of excretion from the body. The GI tract accounts for the largest quantity of lost Zn as a result of unabsorbed dietary Zn and endogenous secretions containing Zn. Since the amount of these secretions varies with dietary Zn intake, endogenous fecal losses can range from < 1 mg/day with low Zn intake to > 5 mg/day with liberal Zn intake (10,55). A second variable route of Zn excretion is losses via sweating which are estimated at 0.5-0.8 mg/day under non-exercising conditions (46,50,56,107). With strenuous exercise, surface Zn losses may reach 1.5 mg/day or 10% of the RDA for Zn intake (27,56,64,69).

Urinary Zn excretion is also variable and subject to a variety of influences. Under normal conditions, urine-Zn losses range from 400-600 μg/day but may be influenced by extremely low or high dietary Zn intake (141). The total amount of Zn excreted is highly correlated with the volume of urine and creatinine excretion. Urine-Zn is primarily from the 2-3% ultrafiltratable portion of plasma-Zn and, under non-exercising conditions, up to

95% of filtered plasma-Zn is reabsorbed (1,20,122,141). Higher urine-Zn losses have, however, been reported for physically training compared to non-training individuals (24,82).

Biological Functions

Zinc provides a variety of biochemical and physiological functions in the body. It is recognized as a component of numerous metalloenzymes serving catalytic, structural and regulatory roles maintaining normal metabolism of CHO, lipids and protein (6,96,137) More than 100 enzymes require Zn, including CA-I, LDH and SOD (4,21,45). The role of CA-I in maintaining acid/base equilibrium in the tissues is vital to the exercising muscles (31,32,37,48,93,97,121). Zinc is also required at the catalytic site of CA-I for optimal enzyme function (69). Likewise, Zn holds a dual purpose in LDH as both a structural and catalytic site component to regulate energy expenditure in glycolysis by catalyzing the reversible reaction between pyruvic acid and lactic acid (31,32,37,80). Sizable amounts of LDH are found in muscle fibers of endurance athletes and prior data substantiates the importance of Zn in this enzymatic role for muscle performance and resistance to fatigue (6,7). Cytosolic SOD, the free-radical scavenger antioxidant enzyme, also requires Zn for structural integrity (58,69).

The structure and function of cellular biomembranes critically rely upon Zn for stability. A loss of Zn from membranes has been shown to increase susceptibility to oxidative damage (66,147). Several researchers conclude that Zn is essential for

maintaining cellular immune response (16,23,35,106,117). Zinc is also necessary for synthesis of nucleic acids and protein metabolism (42,64,89). With critical and diverse biochemical and physiological functions, it is possible that Zn deficiency could impair the capacity to exercise.

The hallmarks of Zn deficiency relate to the array of integral functions Zn fulfills in the body. Notable consequences of deficient Zn status include anorexia, impaired wound healing, compromised immune functions and impaired glucose tolerance (18,64). Abnormal glucose tolerance may be associated with impaired insulin response and with modified lipid metabolism (64,100). Low plasma-Zn levels are linked to altered immunity and increased susceptibility to infection (42,117,120). Plasma-Zn values < 10.7 μmol/L have been used as an indicator of Zn deficiency (105). The occurrence of Zn deficiency in normal healthy adults would seem unlikely with regard to tight homeostatic regulation that decreases total body Zn losses when dietary intake is low. However, when the demand for Zn is elevated, concurrent with an inadequate dietary Zn supply and increased losses, the potential for mild to moderate deficiency becomes more feasible.

Assessment

Plasma-Zn concentration is the most widely used index of Zn status in published research studies (6,22,63,73,87,93,96,120). However, its utility is commonly maligned for lacking sensitivity and specificity as an indicator of Zn status (22,63,106,122). Prior researchers have noted that plasma-Zn is inadequate to detect reductions in dietary intake

or changes in whole body Zn content that would result in marginal or mild Zn deficiency status (21,64,127). Plasma-Zn has been shown to resist a decrease in concentration until dietary Zn intake is so low that homeostasis cannot be reestablished without subsidizing Zn from the exchangeable body pool. It is suggested that this body Zn pool is quite small and is not appropriated until dietary Zn intake subsides below 5 mg/day (63). Since plasma-Zn is a component of the labile Zn pool, some investigators consider it to be a useful and valid indicator of the magnitude of the total body Zn pool; any decrease in plasma-Zn reflects a loss of Zn stores and may preceed an increased risk for metabolic signs of deficiency (63,73). Conversely, other researchers dissent that plasma-Zn reflects body Zn status (15,44,132).

Conditions unrelated to Zn status may cause plasma-Zn to decline. Factors related to depressed plasma-Zn concentrations are inflammation, infection and disease, as well as, stress (21,42,63,122). Plasma-Zn values can be modified by meals, acute fasting, circadian variation, hormone status, alteration of circulating transport protein concentration and muscular lysis (22,63,96,122). Researchers suggest that these conditions, excluding fasting, may provoke a redistribution of Zn from the plasma to other tissues in response to a metabolic demand; thus, manifesting a decrease in plasma-Zn status (63,73). A single, specific and sensitive biochemical index of Zn status is currently unavailable (36,69). Therefore, despite the preceding factors, many researchers continue

to determine, monitor and report Zn status by evaluating plasma-Zn concentrations, often in combination with another biochemical indicator (6,13,22,26,77,110,118-122).

Adjunct biochemical indices can help elucidate Zn status. Erythrocyte-Zn is a circulating Zn reservoir that can be used for assessing Zn status however, few researchers have used it since the analysis can prove difficult (24,26,36,59). Similar to plasma-Zn, the concentration of Zn in erythrocytes can be an insensitive measure of body Zn status (70). Urine-Zn concentrations are appropriate measures only in healthy subjects since disease states can dramatically alter Zn excretion rates. Adults adjust to low Zn intakes and/or the need to conserve tissue Zn levels by decreasing the excretion of this trace mineral (36,63). Twenty-four hour urine collections are preferred since urine-Zn excretion is subject to diurnal variations (36,101,110). Singular assessments do not accurately reflect Zn status, hence a combination of biochemical analysis in conjunction with dietary intake assessments provides the best comprehensive picture of individual Zn status (36,70).

Effects of Aerobic Exercise on Zinc

Research generally indicates that aerobic exercise is a physical stress that alters Zn status, but how Zn metabolism is affected by acute exercise and degree of training is not yet clearly delineated (5,6,20,45). Zinc is presumed to be redistributed within the body both during and after exercise (20,43,45,73). Some investigators report that changes in both urine-Zn and plasma-Zn concentrations, in response to acute exercise, are independent of training status in physically trained and untrained men (6,15). Yet the

reported changes in Zn status are variable with regards to exercise type, intensity and duration, as well as, the timing of the collected sample (3,4,14).

Acute exercise appears to have a transient effect on blood Zn concentrations. Immediately after strenuous exercise, plasma-Zn levels have been reported to significantly increase (5,20,73,93,138). However, under similar conditions, other researchers have reported no significant change in plasma-Zn levels (2,77). Aruoma et al. observed both rises and falls in plasma-Zn immediately after 30-40 minutes of hard cycle ergometer exercise (8). A rapid drop in plasma-Zn concentration has been reported between 30 and 120 minutes or longer after exercise (2,5,13,73,94). Fewer reports are available examining the effects of exercise on erythrocyte-Zn values (72). Concurrent with the immediate post-exercise increase in plasma-Zn levels, a decrease in erythrocyte-Zn concentration has been reported. The concentration of erythrocyte-Zn rebounded to pre-exercise levels after 30 minutes of rest (94,97).

The mechanism responsible for blood Zn concentration changes has not been firmly established though many are suggested. The magnitude of the plasma-Zn increase is not explained as simple hemoconcentration; rather this noted elevation is considered by some researchers to be the result of muscle catabolism that allows released Zn to enter the extracellular fluid (47,73,93,144). The amount of available Zn due to muscle damage may be substantial (21). Nosaka and Clarkson suggest that erythrocyte-Zn, which is more than 10 times higher in Zn content than skeletal muscle, undergoes intravascular hemolysis and

releases Zn into the plasma (87). Alternatively, the increase in plasma-Zn may be a shift from erythrocyte-Zn, which declines immediately post-exercise, mobilizing Zn to plasma in response to the stress of acute exercise (6,72,130). Strenuous exercise has been shown to stimulate an acute-phase response. The resulting increase in serum cortisol concentration is associated with a significant decrease in plasma-Zn concentration (35,45,60). The erythrocyte-Zn loosely bound to CA-I may also be a significant portion of the mobilizable Zn pool swiftly exchanging Zn with plasma (96). The rapid post-exercise fall in plasma-Zn values appears to be related to a shift in Zn from the plasma to the liver and, concurrent with, increased urine-Zn excretion (14,61,102).

Reports of urine-Zn excretion after acute exercise are scant. Anderson et al. (5) observed elevated urine-Zn concentrations, whereas more recent work reported no changes (6). Urine-Zn excretion as SOD was reportedly associated with a decreased plasma level of the enzyme during and after two hours of soccer training (92). Increased Zn losses from acute exercise-induced muscle catabolism may contribute to increased urine-Zn losses and approximate 0.4 mg Zn/day after a single endurance exercise bout (5,24,82).

Conclusions about the alteration of plasma-Zn levels due to chronic exercise between trained and untrained subjects are equivocal. The athletes involved in the majority of these studies have been runners, triathletes, swimmers and collegiates representing a variety of conference sports. Several investigators have demonstrated no

significant difference between physically trained and untrained subjects (24,71,72). Nor was any difference in plasma-Zn levels found when collegiate athletes reported dietary Zn intakes of at least 70% of the RDA (69). In contrast, even when RDA amounts of dietary Zn were consumed some athletes would demonstrate relative hypozincemia (6,118,119).

Generally, physical exercise is associated with depressed resting plasma-Zn values for trained versus untrained control subjects (22,27,43,73,77,96). Specifically, long-term endurance training for male athletes has been shown to decrease resting plasma-Zn values and some researchers note that the relative decrease in plasma-Zn levels is inversely related to the training intensity and total distance logged (27,43,67,77).

The literature offers possible explanations for decreased resting plasma-Zn levels in athletes. 1) The athletic training diet may yield low Zn intake since athletes have been shown to consume less Zn per calorie ingested than did non-exercising control subjects (24,27,43,69,121). 2) Exercise may promote excessive Zn loss since increased sweat rates and endogenous losses of Zn may impact on plasma-Zn levels (5,6,8,82,120). Strenuous running has been associated with urine-Zn losses ~ 1 mg/day, the approximate equivalent of 6% of the RDA for Zn intake (70). Various investigations indicate significant increases (~ 50%) in urine-Zn excretion on the day of strenuous exercise compared with the preceding non-exercise day (5,24,69,138). Serum levels of the stress hormone, cortisol, increase with the intensity and duration of exercise and increased cortisol levels are positively correlated with urine-Zn losses (3). Increased muscle

degradation may lead to increases of urine-Zn that subsequently diminishes resting plasma-Zn concentration (22,70). 3) Expansion of the plasma volume during training could dilute the concentration of plasma-Zn. Investigators agree that this may be a contributing factor to decreased plasma-Zn concentrations, however do not credit it as the cause; contradicting rationales support this consensus (43,45,70).

4) Lastly, a redistribution of plasma-Zn to other tissues may occur. Low plasma-Zn values associated with physical training may involve a shift of Zn to erythrocyte or to the liver for increased synthesis of metallothionein and other circulating Zn transport proteins (11,43,45,67,102). Haymes concludes that plasma-Zn redistribution to other tissues would be apparent when a significant decrease in plasma-Zn concentration was concurrent with no increase in urine-Zn excretion (45).

Copper

Distribution in the Human Body

In comparison with Zn, the human body contains much smaller amounts of Cu. The average adult male possesses approximately 70-80 mg of Cu within body tissues and blood (8,69). Skeletal muscle is the largest depository comprising nearly 25%, with 19% in the skeleton, 15% in both skin and bone marrow, 8-15% in the liver and 8% in the brain (36). Circulating Cu is distributed somewhat equally between the plasma and erythrocyte fractions. Primarily transported by plasma proteins, more than 90% of plasma-Cu is incorporated into ceruloplasmin. The remaining quantities of plasma-Cu are reversibly

bound to albumin, contained in circulating cuproenzymes and attached to amino acids (6,53,109). This ratio appears relatively constant within an individual but varies widely among individuals (135). Approximately 75% of erythrocyte-Cu is bound to cytosolic SOD (78). Normal values for erythrocyte-Cu and plasma-Cu are 10.5-17.5 μmol/L and 11.0-22.0 μmol/L, respectively (134,148).

Diet and Bioavailability

Plasma-Cu values are reportedly insensitive to dietary intakes (118,135). Singh et al. found that only 1% of study subjects demonstrated plasma-Cu values < 11.0 μmol/L when 37% of the subject group had Cu intakes less than the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for Cu (18,118). The ESADDI for adult males is 1.5 - 3.0 mg/day (24-47 μmol/day) (18). However, estimates of the average intake for this population group ranges from < 1.0 - 1.4 mg/day, with 1.2 mg/day reported most frequently as the usual intake (45,51,101,103,134). Published nutrient densities for physically active men range from 0.39-0.67 mg Cu/1000 calories (51,71,101,118,120). Singh et al. observed that dietary Cu intake was significantly correlated with calorie and protein intakes; thus, higher calorie intakes typical of athletic males should allow them to attain ESADDI levels (36,118). The few such reports of dietary Cu intake showed inadequate (120) or adequate (71,115,118,119) ESADDI levels.

The foods highest in bioavailable Cu contain between 0.3 to > 2.0 mg/100 g, such as oysters and the bran and germ portions of grains. See Table 2.2. Cow's milk is a notably poor source of dietary Cu (45,91).

Table 2.2 Copper Content of Major Food Sources of Copper (in mg Cu/100 g)

High (0.3-2.0+)	Intermediate (0.1-0.3)	Low (< 0.1)
Oyster	Grain products	Chicken
Shellfish	Chocolate	Fish
Liver	Dried fruits	Dairy products
Nuts	Mushrooms	Other fruits
Peanut butter	Tomatoes	Other vegetables
Seeds	Banana	
Cocoa powder	Grapes	
Legumes	Most meats	
Whole grains		

Dietary Cu is involved in several nutrient interactions that affect its bioavailability and metabolism. Large quantities of ingested Zn, which is physiochemically similar to Cu, provoke an antagonism which limits Cu absorption. While supplemental doses $\geq$ 1.5 the RDA for Zn are discouraged for potentiating an absorptive competition which can induce Cu deficiency (30,45,86,134,136), dietary intakes of 18.5 mg of Zn have also been shown to limit Cu absorption in young men (28). This mutual antagonism is most apparent when the dietary intake of both trace elements is markedly different (21). As the amount of

dietary Zn increases, the amount of dietary Cu required to maintain Cu balance increases and Zn:Cu ratios $\geq$ 16 may invoke detrimental effects (112). Copper metabolism may also be negatively affected by excessive amounts of iron (78,144), phytate (134), calcium (124), phosphorus (124), and supplemental ascorbic acid (78,134).

An interaction between dietary Cu and the macronutrients may exist (29,108). Carbohydrate as simple sugars, such as fructose or sucrose compared with starch, appears to raise the Cu requirement threefold. Protein intake generally improves Cu absorption (36,112,124). Other researchers, however, observed that large amounts of meat protein may reduce Cu retention (54) or that dietary protein does not affect Cu status (17).

Many factors claim an interaction with Cu however, the actual amount of dietary Cu seems to be more influential on its bioavailability than does the composition of the diet. In two separate studies by Turnlund et al. Cu absorption was altered in response to the amount of Cu ingested (134,135). Slightly more than 40% absorption was associated with a 1.44 mg/day Cu intake which proportionately declined to 25% with a 3.26 mg/day Cu load. Still, the absolute amount of Cu absorbed remained relatively similar. This adaptive mechanism may protect against Cu deficiency or toxicity. Despite factors that may compromise the bioavailability of Cu and the prevalence of suboptimal dietary intakes, Cu deficiency is not observed in adults under normal conditions (86,135).

Metabolism

The absorption of Cu into the GI lumen occurs primarily in the small intestine and is largely controlled by metallothionein in the mucosal cells (112). Most of the absorbed Cu will ultimately be excreted via the GI tract chiefly in bile or in secretory fluids and mucosal cells. Endogenous Cu excretion is inversely proportional to dietary Cu intake. Homeostatic regulation of Cu is maintained by adjusting absorption and excretion via the GI tract (134,135).

Copper can be excreted from the body by two additional routes. Surface losses of Cu via sweat and integumentary chafing account for < 100 μg/day (56,134,135). The purpose of a study by Jacob et al. was to evaluate Cu losses for men living under controlled conditions on a metabolic unit. They estimated surface losses of 0.33 mg/day to elevate the dietary Cu requirement by 25-30% (56). Despite that singular study, researchers are equivocal regarding the ability of sweat Cu losses to contribute significantly to Cu balance (6,71,134,135). In contrast, investigators are in accord that urine-Cu concentrations are very low, with reports ranging from 0.02-0.50 μmol/day (6,118, 127,134,135). Higher urine-Cu values reported prior to 1990 may have resulted from contamination and less reliable analytical methods than currently available. Since trace amounts of Cu are excreted in the urine, this route does not appear to be under homeostatic regulation nor affected by dietary Cu intake (135).

Biological Functions

Copper is a critical nutrient involved in most phases of energy production and/or utilization. It is essential for many physiological functions including erythropoiesis, oxidative phosphorylation, antioxidant protection, and glucose metabolism (126,134,146). Many of these physiological aspects are related to the fundamental role of Cu as a cofactor in numerous enzymatic reactions. As a constituent of cytochrome c oxidase, it permits the terminal step in mitochondrial adenosine triphosphate (ATP) energy production (85). Lysyl oxidase requires Cu for the cross-linking of collagen and elastin which are important for the maintenance of connective tissue and blood vessels (98). Heart rate and blood flow are affected by the catecholamine, norepinephrine, which is synthesized in Cu dependent enzyme reactions (48,97).

Ceruloplasmin, also known as ferroxidase, performs dual key roles. Indirectly, it affects energy metabolism by regulating iron transport and utilization (70,89). As a physiologically active antioxidant, ceruloplasmin, also accomplishes a significant portion of plasma defense against tissue damage by binding Cu and oxidizing ferrous iron (8,109). Similarly, the cuproenzyme SOD is a free radical scavenger which is catalyzed by Cu and present within most cells of the body (45,71). Other roles for Cu are recognized although the mechanisms of Cu involvement are not fully delineated; examples are the maintenance of immunocompetence, glucose and cholesterol metabolism and cardiac function (117, 134). It is obvious that small amounts of Cu play crucial roles in the body.

Assessment

Methods used to determine Cu status include measurements of blood concentrations of Cu and the activity of selected cuproenzymes. Circulating Cu concentrations, however, are not necessarily a reliable index of marginal Cu status and may be applicable only in health and resting conditions (36,86,137,146). Limitations include insensitivity to marginal Cu status and dietary intake and the influence of unrelated factors such as hormone levels, stress and infection. In research studies interested in Cu status, plasma-Cu concentration is used extensively as one chosen parameter (6,8,27,71,72,77, 91,105,109,118-121,126,135,143). Minimal data are available on erythrocyte-Cu concentrations, but some evidence exists that Cu may be redistributed between the plasma and erythrocyte under certain conditions (77,118,146). A seldom used index of Cu status is urine-Cu; excretion levels are very low in healthy subjects due to efficient renal reabsorption (36). Several researchers have used urine-Cu excretion to investigate changes in Cu losses under various conditions (6,15,118).

Effects of Aerobic Exercise on Copper

Relatively few research studies have attempted to determine the influence of physical training and acute exercise on Cu status. Available literature offers conflicting results as to the effects of exercise on circulating and excreted Cu. The variable results reported may reflect the type, duration and intensity of the exercise, in addition to the

training level and dietary Cu status of the subject. Several researchers have found resting blood Cu values to be higher in aerobically trained vs untrained subjects (8,14,19,72,91, 95,121). Yet other investigators have shown no difference (6,27,71,109) or lower circulating Cu values (12,77). Lukaski et al. demonstrated in nationally competitive young adult swimmers that Cu status is not adversely affected by physical activity over a training season when dietary intake of Cu was $\geq$ 67% of the ESADDI for Cu (71).

Elevated circulating Cu levels in athletes may be explained by the functions of Cu that are directly associated with exercise. A biochemical adaptation of aerobic training, which places high demands on energy production and the mitochondrial cuproenzyme, cytochrome c oxidase, may occur to raise plasma-Cu concentrations. Alternatively, the role of Cu in ceruloplasmin for iron transport and antioxidant function may be responsible for higher plasma-Cu values (45,72,85,89). Altered Cu metabolism and tissue redistribution may be an adaptive consequence of exercise, as well (71,77,121).

Similarly, acute exercise has led to reports of variable changes in circulating Cu levels (5,91,95,109). Recently, Anderson et al. demonstrated that, in male subjects performing acute exercise at 90% VO_2 max which continued to exhaustion, plasma-Cu concentrations were highly significantly increased at cessation of exercise (6). Similar results were shown in previous studies using strenuous cycle ergometer exercise lasting 30-120 minutes (91,95). Anderson et al. further showed that plasma-Cu values returned

to baseline levels two hours post-exercise and that this phenomenon was independent of training status in moderately trained vs. untrained subjects (6,19).

In contrast, Bordin et al. exercised subjects at 80-90% VO_2 max on a treadmill and found that plasma-Cu concentrations decreased more than 50% from baseline values (13). Within 30 minutes after exercise plasma-Cu values in this study group returned to pre-exercise levels. Marrella et al. worked healthy triathletes at 70% VO_2 max on a cycle ergometer and evaluated the plasma-Cu response over an extended post-exercise time frame (77). Their results showed a significant exercise-induced decrease in plasma-Cu immediately after exercise which returned to baseline values within one hour. At two hours post-exercise plasma-Cu had again decreased slightly, but significantly, from baseline values. A prior study by Aruoma et al. observed a mean decrease in plasma-Cu levels after 30-40 minutes of strenuous cycle ergometer exercise (8). They recorded an individual response to exercise for 17% of subjects with increased, and 17% with no change in, plasma-Cu values. Other researchers have demonstrated no significant change in plasma-Cu concentrations in response to acute strenuous exercise (5,119,120).

Few studies have evaluated erythrocyte-Cu in relation to exercise (72,121) although some protocols have used total blood cell Cu concentrations (77) or erythrocyte-SOD activity (58,71,135). The erythrocyte-Cu content includes that bound to SOD and contributes to total blood cell Cu. Singh et al. observed that female runners had significantly lower erythrocyte-Cu levels than did non-runners (121). In male athletes,

Lukaski et al. showed that erythrocyte-Cu and plasma-Cu concentrations were significantly positively correlated; based on significantly higher plasma-Cu levels, they implied that erythrocyte-Cu was also significantly higher in trained compared to untrained subjects (72). Marella et al. recorded that total blood cell Cu was higher in male athletes vs non-athletes and did not change in response to acute strenuous exercise (77). Reports are lacking which examine erythrocyte-Cu changes in response to acute exercise in male athletes.

Reports of urine-Cu associated with exercise are sparse (6,118). Anderson et al. reported no changes in urine-Cu losses after acute exercise but noted that the duration of physical activity may have been insufficient to elicit a significant difference (6). Altered urine-Cu losses observed under physical training conditions may be associated with the duration and strenuousness of the exercise (4-6).

Strenuous acute aerobic exercise results in many metabolic changes that impact an athlete. The review of numerous research studies suggests variable alterations in plasma, erythrocyte and urine concentrations of Zn and Cu up to two days post-exercise and a potential for inadequate dietary intake of Zn and Cu in physically active males.

CHAPTER III

MATERIALS AND METHODS

Subjects

Twenty well-trained male cyclists were selected to participate in this study. Potential subjects were recruited by telephone from team racing lists obtained from area cycling clubs and university athletic departments. The cyclists were selected on the basis of their current training schedule (average 10-15+ hr/wk peak training season), competitive racing status (designated category 2=semi-professional to 5=novice in road cycle racing and/or E=expert or S=sport in off-road cycle racing according to the United States Cycling Federation (USCF) definitions) (Appendix A), age (18-36 years old), and that they had not taken any dietary mineral supplement for the one month prior to the initiation or duration of this study. All participants signed an informed letter of consent after being told the limited risks associated with this study (Appendix B) and completed a medical and physical training questionnaire (Appendix C). One subject dropped out during the study due to work schedule conflicts.

Experimental Design

Exercise

The study required each subject to perform an anaerobic threshold (AT) determination test on a cycle ergometer (CompuTrainer) according to the Conconi

Method (Appendix D) at least three days prior to the start of the exercise protocol. Using the same equipment, each subject exercised at 80-90% of AT for 60 minutes, after which, work intensity (watts) was increased by 20 watts every minute until the subject reached voluntary exhaustion. See Table 3.1

Table 3.1 Study Protocol Schedule

Day	Description	Blood Draws	Urine Collection	Diet Record
	AT Test performed 3 days prior to exercise day	-	Instructions Given	Instructions Given
1	Day Before Exercise	-	BASE	Day 1
2	Exercise Day	PRE PE PE-2H	EX	Day 2
3	1 Day Post-Exercise	PE-1D	PE-1	Day 3
4	2 Days Post-Exercise	-	PE-2	Day 4
5	3 Days Post-Exercise	PE-3D	-	Day 5
6	4 Days Post-Exercise	-	PE-4	Day 6
7	5 Days Post-Exercise	PE-5D	-	-

Blood Collection

Blood was collected at six specified time intervals: A) prior to the exercise protocol (PRE), B) immediately upon cessation of the exercise (PE), C) two hours (PE-2H), D) one day (PE-1D), E) three days (PE-3D), and F) five days post-exercise (PE-5D), respectively. See Table 3. Blood samples were drawn from an antecubital vein, into a heparinized 15 ml tube free of trace minerals (Becton Dickinson), using a 1 1/2 inch 21-guage stainless steel needle (Becton Dickinson). The cyclists were asked to refrain from exercise training at > 60% of AT during the five post-exercise sample collection days. Blood samples were centrifuged at 1500x G for three minutes and then at 3500 x G for five minutes (Baxter SP Clinifuge). Plasma was obtained using disposable pipettes and placed in 2 ml polyethylene tubes and stored at -80° C for analysis. Erythrocytes were separated from the buffy layer, rinsed with 9% NaCl solution, repeatedly inverted and recentrifuged according to the prior procedure. The supernatant was extracted and discarded. Erythrocytes were mixed 1:1 with deionized water (DIW) in 2 ml polyethylene tubes and stored at -80° C for analysis. All blood samples were stored immediately and in triplicates.

Urine Collection

The subjects collected 24 hour urine samples the day prior to (BASE), the day of (EX), 2 days after (PE-2) and 4 days after (PE-4) the prolonged exercise ride,

respectively. Subjects who performed the exercise protocol after 6 p.m. collected an additional urine sample one day post-exercise (PE-1) since the EX collection would represent > 12 hours before the exercise protocol. See Table 3.1. Urine was collected in 4L specimen bags (Baxter Scientific Products) and weighed on a 4 kg taring scale (Sartorius) to determine volume. A 10 ml aliquot from each subject collection was placed in 2 ml polyethylene tubes and stored immediately at -80° C until analysis.

Dietary Data

Dietary data consisted of a consecutive six day food diary initiated one day prior to the prolonged exercise ride (Appendix E). One registered dietitian provided 10 minutes of individual instruction to each subject on how to properly complete their diet records. The same dietitian reviewed the diet records for completion and accuracy of all necessary information with each subject on the last day of their participation. See Table 3.1.

All food records were analyzed by the same dietitian using Diet Max software, update 1995 (Positive Input, Inc., Three Rivers, MI). This program provided a database of > 7,000 foods from many sources, including those from the United States Department of Agriculture (USDA), fast-food outlets and labels from commercial, brand-name foods. Nutrient values of Zn and Cu varied $\leq$ 7% from the USDA database. When an exact match between the diet record food and the Diet Max database was not possible, the closest substitution was made. Mean nutrient values for the six day period were obtained

(raw data in Appendix F). Dietary intakes and percent RDA or ESADDI values were calculated for energy and selected macronutrients and minerals.

Biochemical Analysis

Plasma and erythrocytes were analyzed for Zn and Cu by flame atomic absorption spectrophotometry (AAS) using a Varian SpectrAA-40 instrument (Varian Analytical Instruments, Springale, Australia) according to standard procedures (Appendix G and H). Urine-Zn concentrations were also analyzed by flame AAS (Appendix G). Urine-Cu concentrations were analyzed by graphite furnace AAS according to the method provided by Varian (Appendix H).

Statistical Analysis

Analysis of variance (ANOVA) with repeated measures was used to test within the group over time. Biochemical data obtained at each collection were compared to data obtained at baseline (subjects served as their own controls). Two tailed paired t-tests were computed to determine the specific significant difference between collection points. Statistical analysis for ANOVA was performed using the BioMedical Data Package Statistical Software (BMDP-Los Angeles, CA). Descriptive statistics and t-tests were accomplished using Microsoft Excel Analysis ToolPak (GreyMatter International, Cambridge, MA). A $p < 0.05$ was considered significant for ANOVA measures and

$p < 0.01$ was significant for t-tests. Reported values are expressed as: mean $\pm$ standard error of the mean (SEM).

CHAPTER IV

RESULTS

Demographic Data

The 19 healthy cyclists averaged 26.7 ± 1.2 years in age (mean ± SEM), 78.5 ± 2.0 kg in weight, and 177.6 ± 3.8 cm in height. See Table 4.1. Cycling accounted for nearly all of their training time (mean training 15.5 ± 2.5 hours/week) and they had a mean AT of 183.5 ± 2.5 beats/minute (range: 163-199). At exhaustion, subjects had cycled a mean of 67.3 ± 0.9 minutes. All racing categories were represented: 2 (n=3), 3 (n=5), 4 (n=8), 5 (n=1), E (n=1), and S (n=1).

Table 4.1 Demographic Data of Study Subjects

	(a) Category	Age	Height (cm)	Weight (kg)	(b) AT	(c) Hours per wk	(d) Exercise Time
Subject 1	E	18	175.0	65.9	196	20	68
Subject 2	2	23	187.5	75.0	176	35	65
Subject 3	4	34	180.0	79.5	185	18	66
Subject 4	4	23	175.0	68.2	169	16	67
Subject 5	4	30	170.0	70.5	163	15	67
Subject 6	3	35	182.5	88.6	177	24	62
Subject 7	4	23	180.0	79.5	180	15	65
Subject 8	4	23	175.0	88.2	197	16	66
Subject 9	3	31	180.0	75.0	187	8	66
Subject 10	4	24	165.0	83.6	192	10	74
Subject 11	2	20	185.0	77.3	189	19	67
Subject 12	5	22	180.0	81.8	180	7	66
Subject 13	3	25	177.5	84.1	196	7	70
Subject 14	4	30	172.5	88.2	190	12	65
Subject 15	4	34	180.0	81.8	168	13	65
Subject 16	2	25	165.0	61.4	199	11	67
Subject 17	S	23	180.0	76.4	193	16	67
Subject 18	3	36	192.5	95.0	169	20	80
Subject 19	3	28	172.5	70.9	192	5	65
Average		27	177.6	78.5	184	15	67

(a) Racing Category: E=Expert, S=Sport, 1=Professional to 5=Novice.
(b) AT is measured anaerobic threshhold.
(c) Hours per week of physical training.
(d) Total exercise protocol time to exhaustion (minutes)

Biochemical Measures

Plasma and erythrocyte concentrations for both Zn and Cu were significantly different ($p<0.05$) over the time period from PRE to PE-5D blood draws (Figures 4.1-4.4, respectively). The PE concentration for plasma-Zn was significantly higher ($p<0.01$) than the PRE, PE-2H, PE-1D, PE-3D and PE-5D values. Plasma-Zn levels at PRE were low with 84% of subjects in the lower half of the reference value range and more than 10% with hypozincemia (< 11.5 µmol/L). By PE-5D, 26% of subjects met the hypozincemia criteria.

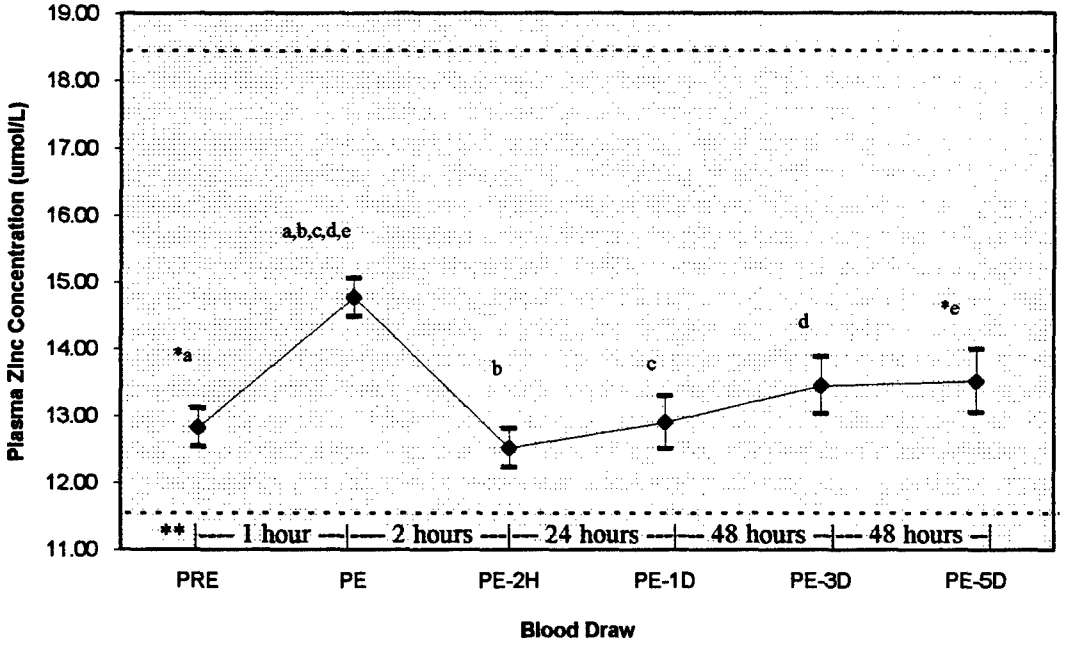

Figure 4.1 - Plasma Zinc Concentration. Area between horizontal lines represents normal reference value range. * A significant difference ($p<0.05$) over time between PRE and PE-5D. ** Hours represent time intervals between blood draws. a,b,c,d,e - A significant difference ($p<0.01$) between blood draws. PRE=pre-exercise, PE=post-exercise, PE-2H=2 hours post-exercise, PE-1D=1 day post-exercise, PE-3D=3 days post-exercise and PE-5D=5 days post-exercise. N=16.

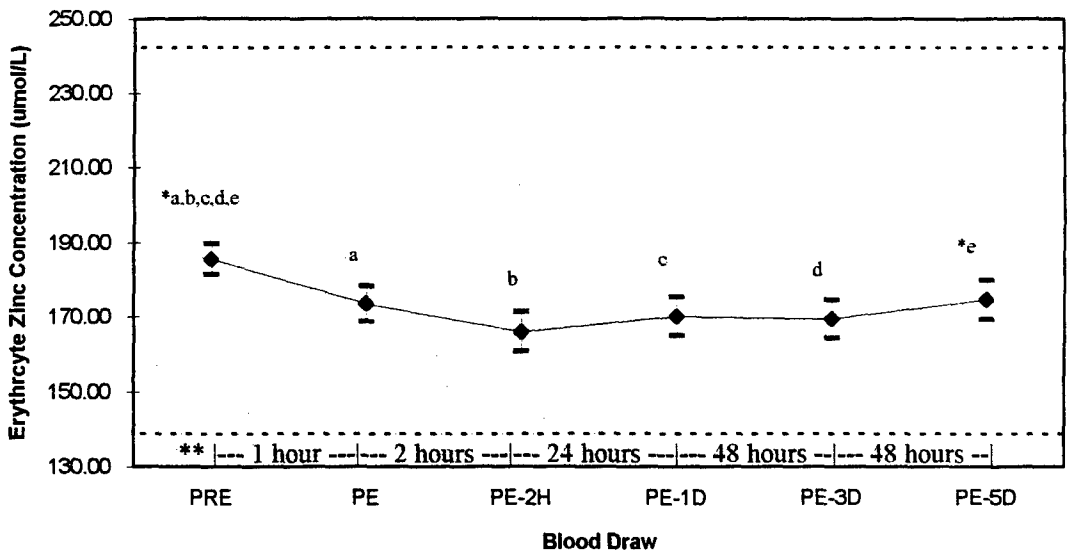

Figure 4.2 - Erythrocyte Zinc Concentration. Area between horizontal lines represents normal reference value range. * A significant difference ($p<0.05$) over time between PRE and PE-5D. ** Hours represent time intervals between blood draws. a,b,c,d,e - A significant difference ($p<0.01$) between blood draws. PRE=pre-exercise, PE=post-exercise, PE-2H=2 hours post-exercise, PE-1D=1 day post-exercise, PE-3D=3 days post-exercise, and PE-5D=5 days post-exercise. N=16.

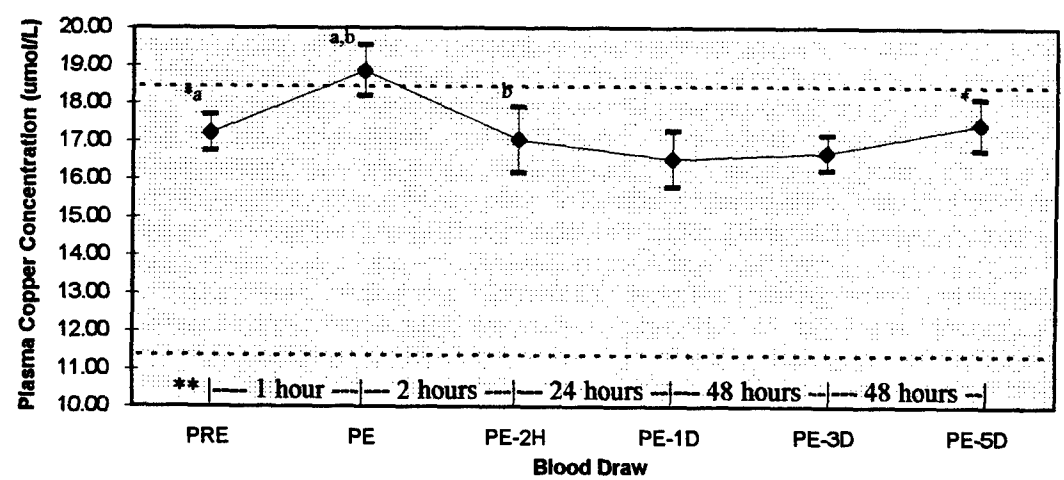

Figure 4.3 - Plasma Copper Concentration. Area between horizontal lines represents normal reference value range. * A significant difference ($p<0.05$) over time between PRE and PE-5D blood draws. ** Hours represent time intervals between blood draws. a, b - A significant difference ($p<0.01$) between blood draws. PRE=pre-exercise, PE=post-exercise, PE-2H=2 hours post-exercise, PE-1D=1 day post-exercise, PE-3D=3 days post-exercise and PE-5D=5 days post-exercise. N=16.

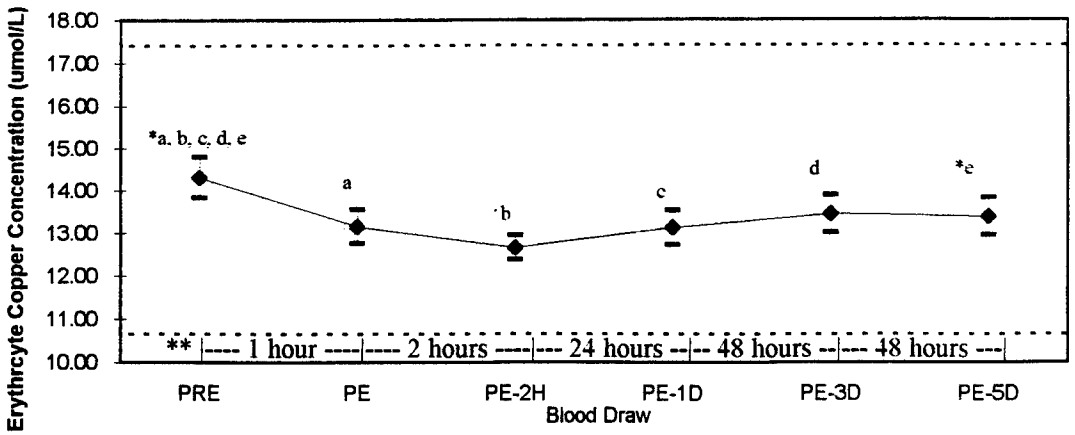

Figure 4.4 - Erythrocyte Copper Concentration. Area between horizontal lines represents normal reference value range. * A significant difference ($p<0.05$) over time between PRE and PE-5D blood draws. ** Hours represent time intervals between blood draws. a,b,c,d,e - A significant difference ($p<0.01$) between blood draws. PRE=pre-exercise, PE=post-exercise, PE-2H=2 hours post-exercise, PE-1D=1 day post-exercise, PE-3D=3 days post-exercise and PE-5D=5 days post-exercise. N=16.

The PRE values for erythrocyte-Zn and erythrocyte-Cu were significantly higher ($p<0.01$) than each subsequent blood draw, respectively. The PRE values for erythrocyte-Zn and erythrocyte-Cu were also within the reference range for 100% and 90% of subjects, respectively. Similarly, 90% of subjects were in the upper half of the reference value range for plasma-Cu values at PRE. The plasma-Cu value at PE was significantly higher ($p<0.01$) than PRE and PE-2H values.

Urine excretion for Zn and Cu were not significantly different at any collection points within or over the testing time period (Figure 4.5 and 4.6, respectively). A slight but non-significant decline in urine-Zn concentration appeared between BASE and EX values. Urine-Cu levels decreased slightly at PE-1 and rose sharply through PE-4 however, none of these alterations were statistically significant.

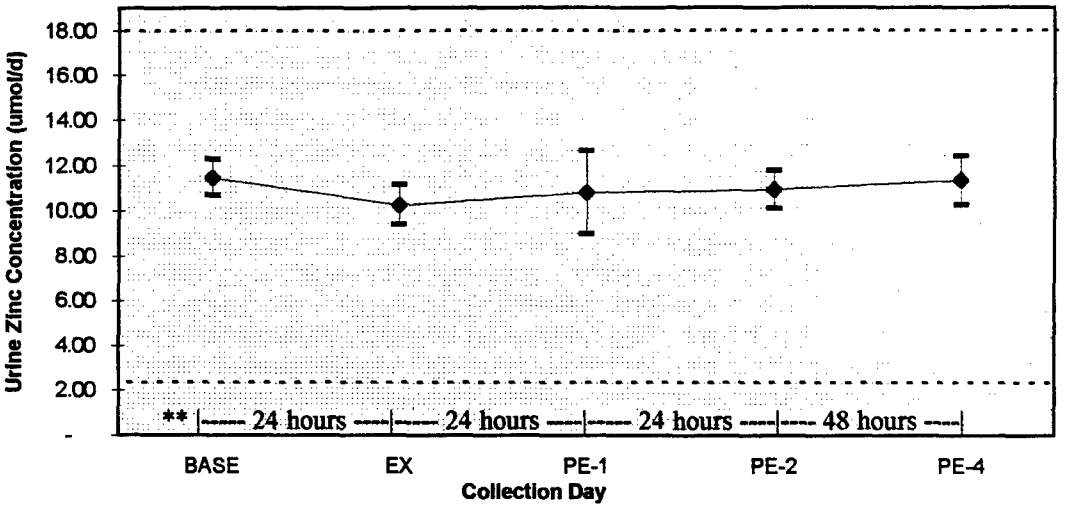

Figure 4.5 - Urine Zinc Concentration. Area between horizontal lines represents normal reference value range. ** Hours represent time intervals between urine collection days. BASE=1 day prior to exercise, EX=exercise day, PE-1=1 day post exercise, PE-2=2 days post-exercise and PE-4=4 days post-exercise. N=7.

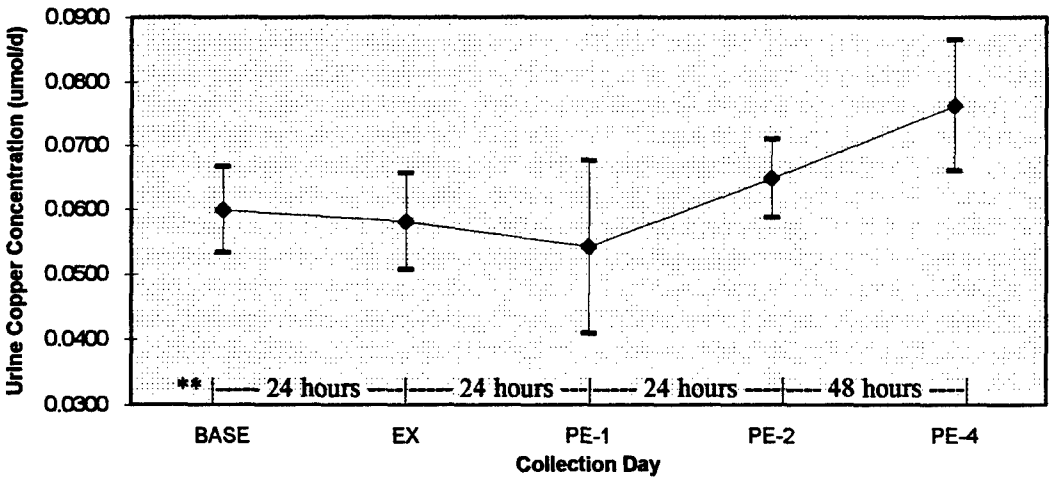

Figure 4.6 - Urine Copper Concentration. Normal value reference range is < 0.630 umol/d. ** Hours represent time intervals between urine collection days. BASE=1 day prior to exercise, EX=exercise day, PE-1=1 day post exercise, PE-2=2 days post-exercise and PE-4=4 days post-exercise. N=7.

Dietary Data

Mean energy intake of the self-selected diets was 3,317 ± 173 kcals/day with the macronutrient distribution of CHO, protein and fat at 56%, 16%, and 28% of total kcals, respectively (Figure 4.7 and 4.8, respectively). Mean dietary Zn intake (13.7 ± 1.1 mg/day) was below the RDA of 15 mg/day (Figure 4.9). Nearly 28% and 78% of subjects had Zn intake < 70% and < 100% of the RDA, respectively. Dietary Zn density averaged 4.1 mg Zn/1000 kcals. See Table 5.

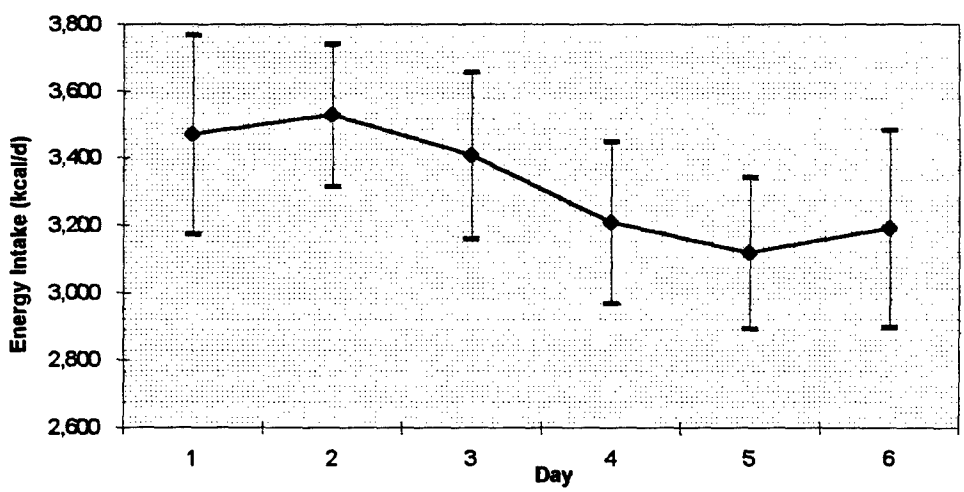

Figure 4.7 - Energy Intake. Values represent means +/- SEM. N=18.

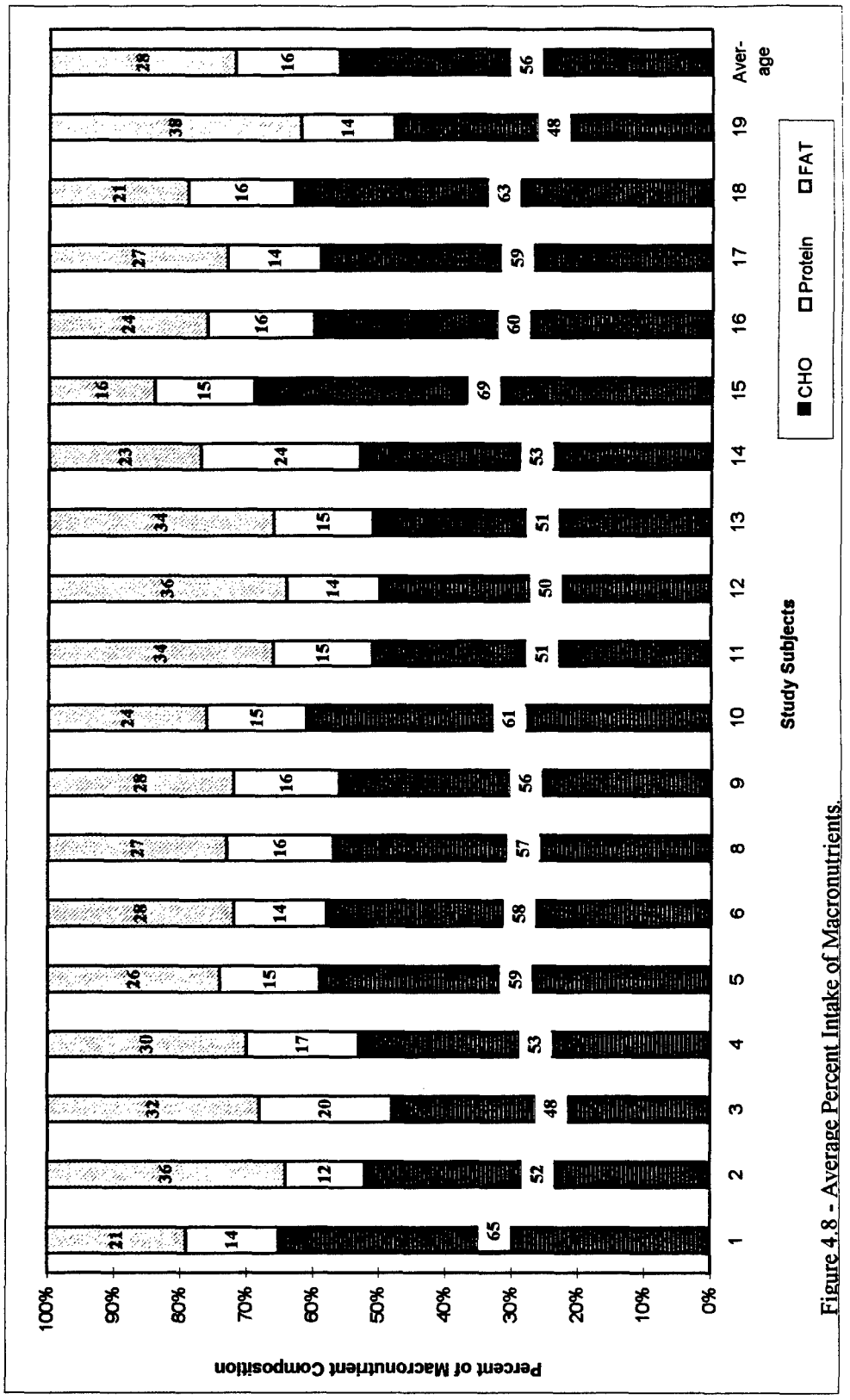

Figure 4.8 - Average Percent Intake of Macronutrients.

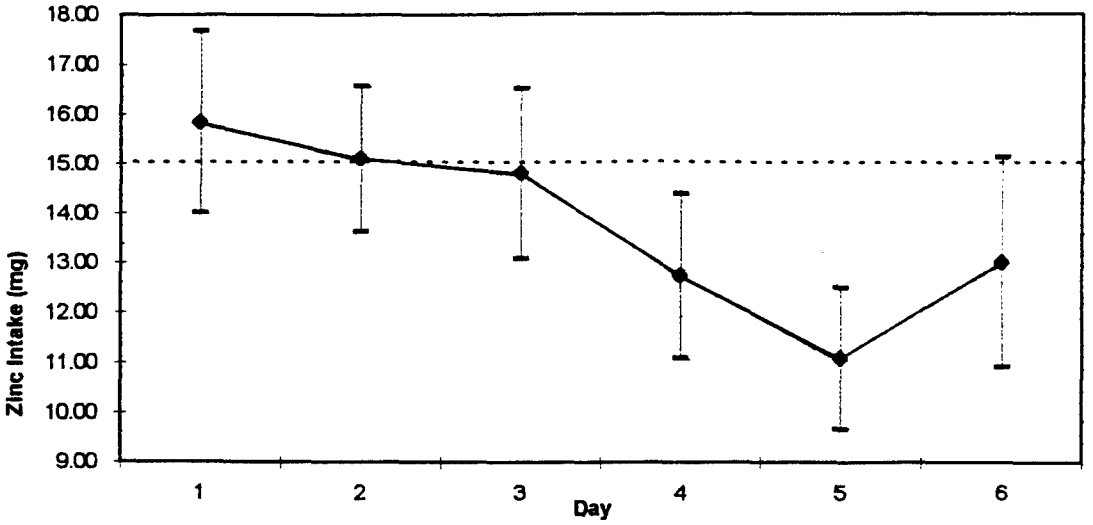

Figure 4.9 - Dietary Zinc Intake. Values represent means +/- SEM. Horizontal line represents RDA for Zinc of 15 mg/day. N=18.

Table 4.2 Trace Mineral Densities. Calculated per 1000 calories. N=18.

	Copper	Zinc
Subject 1	0.4	3.8
Subject 2	0.2	3.2
Subject 3	0.8	5.7
Subject 4	0.5	3.4
Subject 5	0.6	3.8
Subject 6	0.5	4.8
Subject 8	0.6	3.7
Subject 9	0.4	3.1
Subject 10	0.6	4.2
Subject 11	0.5	3.7
Subject 12	0.5	3.7
Subject 13	0.3	3.7
Subject 14	0.5	4.8
Subject 15	0.8	4.3
Subject 16	0.7	5.7
Subject 17	0.3	3.4
Subject 18	0.5	4.7
Subject 19	0.5	4.0
Average	0.5	4.1

Mean dietary Cu intake (1.7 ± 0.1 mg/day) surpassed the lower limit of the ESADDI for Cu of 1.5-3.0 mg/day for only half of the cyclists (Figure 4.10). Slightly more than 11% of subjects did not achieve 70% of the 1.5 mg Cu/day level. Dietary Cu density averaged 0.5 mg Cu/1000 kcals. See Table 4.2

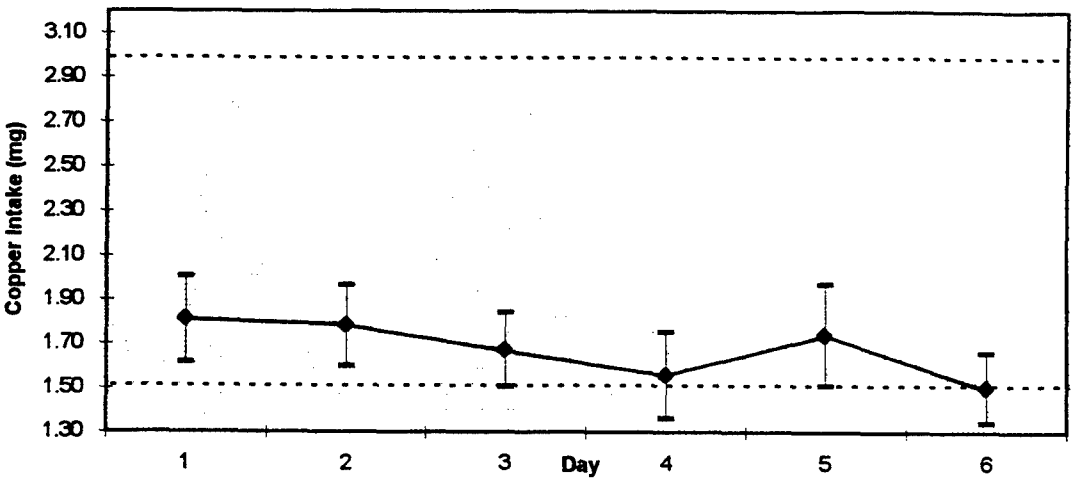

Figure 4.10 - Dietary Copper Intake. Values represent means +/- SEM. Area between horizontal lines represents ESADDI for Copper of 1.5 to 3.0 mg/day. N=18.

CHAPTER V

DISCUSSION

Acute, strenuous aerobic exercise produced highly significant shifts in blood concentrations of Zn and Cu in the present study. The PE increase in plasma-Zn was highly significant ($p<0.00001$) and is consistent with the results obtained by previous researchers under similar exercise conditions (5,6,13,73,93,138). In a recent report by Anderson et al. (6), subjects were moderately trained men with plasma-Zn levels slightly higher at PRE and nearly matched with those of the present study at the PE and PE-2H concentrations. In contrast, Auroma et al. (8) observed that different athletes exposed to comparable exercise stress showed markedly different changes in plasma-Zn; only half of their subjects who rode a cycle ergometer at 80% $V0_2$ max for 40 minutes had increased plasma-Zn levels at PE. Other subjects in the Aruoma study showed no change in plasma-Zn concentrations in response to acute exercise, which was in accord with the results of some investigators (2,77,120,122). The equivocal results reported on the initial change in plasma-Zn may relate to differences in the duration, type and intensity of the acute exercise, as well as the training level and individual response of the subjects (3,4,8,14).

Considerable agreement has been shown in identifying a significant and rapid decrease in plasma-Zn levels between 30 - 120 minutes after the cessation of acute, strenuous exercise (2,5,6,13,73,93). In the present study, the decline in plasma-Zn at PE-2H was highly significant ($p<0.00001$). Concurrent with the alterations in plasma-Zn

between PRE and PE-2H, was a steady, highly significant decline ($p<0.00001$) in plasma-Zn concentration (Figure 5.1). Though the initial decline in erythrocyte-Zn during exercise is consistent with prior reports, other investigators observed that concentrations returned to PRE levels within 30 minutes of PE (20,93,97).

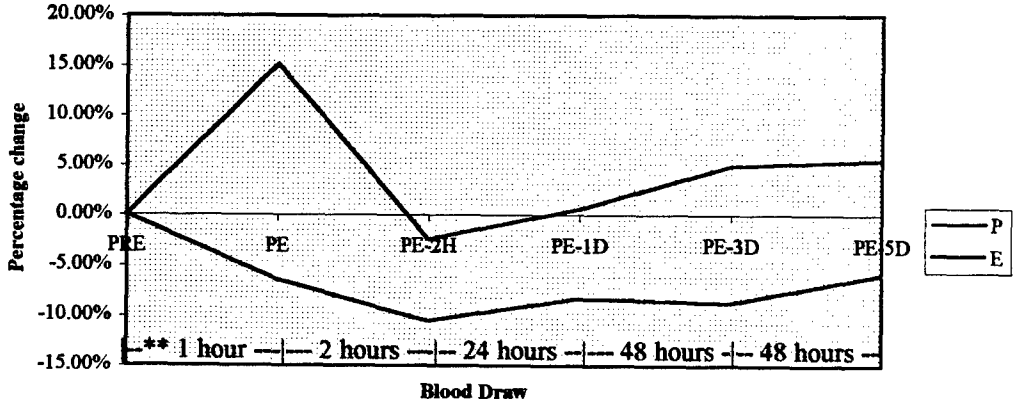

Figure 5.1 - Plasma and Erythrocyte Zinc Changes. The X axis represents the PRE tested levels for both Plasma and Erythrocyte. The testing points represent the percentage change from the PRE levels. PRE=pre-exercise, PE=post-exercise, PE-2H=2 hours post-exercise, PE-1D=1 day post-exercise, PE-3D=3 days post-exercise, PE-5D=5 days post-exercise. ** Hours represent time intervals between blood draws. N=16.

The alterations in plasma-Zn during arduous exercise may be attributed to a combination of exercise-induced effects. The magnitude of the PE increase for plasma-Zn, as well as plasma-Cu, cannot be explained as a simple consequence of hemoconcentration (6,47,73,93,119). Though typical sweat rates of ~ 1 L/hour during heavy exercise can

before and 1-2 L ad libitum throughout the 60-75 minute exercise protocol. Furthermore, room conditions were set to minimize sweat loss: ambient air temperature was 72.3±0.4 ° F, humidity was 38.9 ± 1.6% and a room fan was directed at each subject. Therefore, the plasma increase of Zn and Cu was likely not the result of changes in plasma volume.

It is more probable that exercise induces a tissue redistribution of Zn (77,118,121). Ohno et al. (93) concluded that erythrocyte-Zn may be regarded as a significant part of the mobilizable Zn pool in response to intense cycle ergometry exercise. They demonstrated that the elevation of plasma-Zn levels may be partly due to a shift of Zn from the CA-I store in the erythrocyte. Since erythrocyte-Zn concentrations also returned to PRE levels in other reports (93,97), then erythrocyte-Zn, as CA-I, appears to be a feasible contributor to increased plasma-Zn values as well as an acceptor of Zn from plasma in dynamic tissue redistribution (20). Exercising muscles vitally depend on CA-I to maintain tissue acid/base balance. A chronic exercise-induced adaptation in Zn metabolism appears to be the general elevation of basal erythrocyte-Zn and parallel decline of plasma-Zn concentration; thus, further supporting the role of erythrocyte-Zn as a mobilizable portion of the Zn pool (90,121).

Other mechanisms mediating redistribution of Zn may occur as a result of tissue damage and inflammation generated by strenuous exercise. Muscular lysis, evident by increased serum myoglobin levels, leaks intracellular Zn into the extracellular fluid, subsequently raising the plasma-Zn concentration (5,20,22,47). Lukaski et al. concluded

that the magnitude of the plasma-Zn increase was positively related to the saturation of the tissue pools (e.g. muscle) as a result of the adequacy of Zn in the diet (73). Subsequently, the transient PE decline in plasma-Zn may be the result of stress response hormones, leucocytic endogenous mediator (LEM) and/or cortisol, that stimulate hepatic sequestration of Zn from circulating Zn pools (6,20,21,102,118,121). Liver and other tissues actively involved in exercise may have a preferential demand for Zn which serves to decrease plasma-Zn levels (6,22,118,119,121). As the duration and intensity of exercise increase, so does physical stress and serum cortisol (6), which may redistribute progressive amounts from plasma-Zn concentrations. This decrease in plasma-Zn concentration is typical of an acute phase response, which is a biochemical and endocrine reaction resulting from the physical trauma and inflammation that follows prolonged strenuous exercise (8,11,15,119). The stresses of acute, high intensity exercise also increase interleukins-1 and -6 which may contribute to decreased plasma-Zn levels (6,119).

The acute post-exercise decrease in plasma-Zn levels is usually associated with increased urine-Zn excretion (5,14,24,43,61,118,121,138). The magnitude of urine-Zn losses is positively related to the level of physical stress and serum cortisol which are both increased with prolonged intense exercise (5,6). Prior researchers have postulated that elevated urine-Zn losses serve to clear the increased plasma-Zn levels resulting from muscular lysis (22,24). The urine-Zn excretion has been shown to be significantly increased (~ 50%) on the day of strenuous exercise as compared to the preceding non-

exercise day (5,24,138). In contrast, the findings of the present study showed a slight, but non-significant decrease on the EX day. This result is congruent with those of other investigators who have found that urine-Zn excretions were similar before and after prolonged intense physical activity performed by young men (6,119,120). The duration and intensity of exercise in the present study was sufficient to provoke urine-Zn losses. These findings indicate that mechanisms other than urine-Zn losses were responsible for the decrease in plasma-Zn. Sweat losses, which can be significant with strenuous exercise, are nonetheless, more apt to influence chronic plasma-Zn levels than the acute PE-2H decline (8,27,56,64,69).

Similar mechanisms may account for the alterations in circulating Cu in the present study (Figure 5.2). The PE elevation of plasma-Cu levels was highly significant ($p<0.001$) and in accord with other studies using strenuous cycling (8,73,91,95) and treadmill running exercise (6). However, the observed initial plasma-Cu response conflicts with some investigators, using similar trained athletes and exercise conditions, who reported decreased (8,13,77) or unaltered (5,8,120) plasma-Cu concentrations at PE. The latter studies involved small groups, each less than 12 subjects. Aruoma et al. found highly individual alterations in the response of P-Cu to strenuous physical exertion (8). When various studies examined plasma-Cu levels within 30 - 120 minutes of PE, concentrations consistently returned to PRE values (6,13,77). Marrella et al. observed a second significant plasma-Cu decrease between the 1 hour and 2 hour measures (77). In the present study, a significant decrease was also recorded for plasma-Cu at PE-2H. The

decrease in erythrocyte-Cu concentration was highly significant from PRE to PE ($p<0.0001$) and persisted to PE-2H. The PE-5D levels of erythrocyte-Cu remained significantly lower than PRE. Prior research that has examined erythrocyte-Cu concentrations has done so with regard to the effects of chronic, rather than acute, exercise.

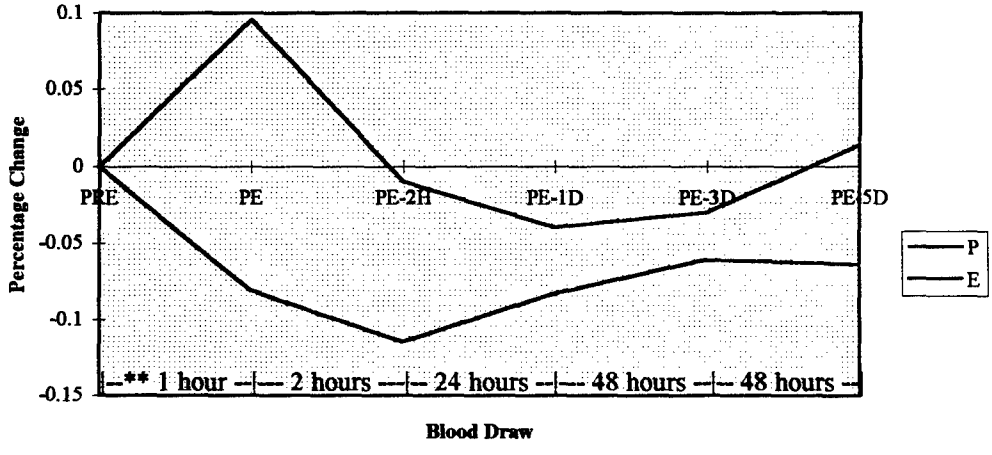

Figure 5.2 - Plasma and Erythrocyte Copper Changes. The X axis represents the PRE tested levels of Copper for both Plasma and Erythrocyte. The subsequent blood draws represent the percentage change from the PRE levels. PRE=pre-exercise, PE=post-exercise, PE-2H=2 hours post-exercise, PE-1D=1 day post-exercise, PE-3D=3 days post exercise and PE-5D=5 days post-exercise. ** Hours represent time intervals between blood draws. N=16.

The decrease in erythrocyte-Cu, largely comprised of superoxide dismutase, may reflect the accelerated activity of this Cu dependent antioxidant enzyme during aerobic exercise (71). An increase in free radical formation is an established consequence of strenuous aerobic activity (45,58,71) and ceruloplasmin, the primary plasma-Cu carrier, is a physiological antioxidant (8,109). During exercise, the rise in plasma-Cu, as

The decrease in erythrocyte-Cu, largely comprised of superoxide dismutase, may reflect the accelerated activity of this Cu dependent antioxidant enzyme during aerobic exercise (71). An increase in free radical formation is an established consequence of strenuous aerobic activity (45,58,71) and ceruloplasmin, the primary plasma-Cu carrier, is a physiological antioxidant (8,109). During exercise, the rise in plasma-Cu, as ceruloplasmin, may be a compensatory mechanism to provide auxiliary antioxidant tissue defense (8,119). Alternatively, increasing plasma-Cu levels may be associated with the roles of Cu in iron transport as a component of ceruloplasmin or in energy production as a component of cytochrome c oxidase (72,89). Exercise-induced muscle catabolism was also shown to elevate plasma-Cu levels (73). When dietary Cu intake was adequate, large increases in plasma-Cu indicated that muscle tissue stores were filled.

Tissue redistribution of Cu is implicated when parallel declines occur in plasma-Cu and E-Cu concentrations. Similar to Zn metabolism, accelerated hepatic synthesis of ceruloplasmin may increase the demand for P-Cu, thereby attenuating these concentrations (11,119). Contrary to decreasing plasma-Zn, an acute phase response typically results in an increase of plasma-Cu (8,11,119). However, in the present study, plasma-Cu levels significantly decreased from cessation of exercise through PE-1D. Similar results have recently been reported (6,119). In addition, sweat losses may also contribute to decreased plasma-Cu levels (8,109) yet, in the present study, plasma-Cu levels increased during peak sweat excretion.

The dietary macronutrient distribution of the subject group approximated optimal athletic nutrition guidelines of > 60%, 12-15% and < 30%, for CHO, protein and fat intakes, respectively (80). The diet records showed a heavy reliance on convenience and fast food choices. Predominant selections were CHO-rich foods, including cold cereal, ice cream, pastries, Gatorade ®, soda, pizza and bean dishes such as burritos and chili. The observed macronutrient proportions were similar to those of a controlled diet designed by Anderson et al. to be representative of normal United States diets (6). In their study, the dietary density of Cu and Zn were 0.48 $\pm$ 0.06 mg Cu and 5.8 $\pm$ 0.2 mg Zn, expressed per 1000 kcals, respectively. As Anderson noted, at these Cu intakes, more than 3000 kcals are needed to meet the minimum ESADDI. The cyclists predominately selected foods which would be considered intermediate sources of Cu. High caloric intake, rather than Cu rich food sources, likely enabled the subject group to achieve > 1.5 mg Cu/day (6,118). Though dietary Cu consumption for 50% of the cyclists was seemingly inadequate, plasma-Cu levels were relatively high throughout the study period with the exception of one subject. Comparably, Singh et al. reported 37% of their subjects with Cu intakes < 2.0 mg/day and only 1% with subnormal plamsa-Cu levels (118). Other athletes with adequate Cu consumption and energy intakes similar to those of the cyclists, were able to maintain acceptable plasma-Cu values (71,115,120). Elevated plasma-Cu concentrations however, are reported to be a physiological adaptation of chronic aerobic exercise and not necessarily a reflection of dietary Cu intake (14,19,72,91,95,121). In a study of non-exercising men, Turnland et al. determined that dietary Cu intake as low as

> 0.8 mg/day was sufficient to maintain acceptable plasma-Cu levels for up to 42 days (135). For strenuously exercising athletes however, more optimal Cu consumption may expedite the return of plasma-Cu and erythrocyte-Cu to PRE levels.

Dietary Zn intakes of the cyclists in the present study were similar to those reported for runners (120), but lower than those of other physically active men (71,115,118,119). The majority of dietary Zn for the cyclists was likely contributed by the cereal, chicken and legume consumption reported in the food records. With > 3000 kcals/day in energy intake, the cyclists would have met the RDA if their Zn density had been within the 5.0-6.0 mg/1000 kcals range reported by others (6,71,101,104). By PE-5D, hypozincemia was evident in 26% of the cyclists. This is comparable to reported hypozincemia in 23% of runners (27) and in other male athletes (43). The premise that diminished circulating Zn levels in endurance athletes may be related to inadequate dietary Zn (20,71) could be supported by the high percentage of cyclists who did not achieve RDA intakes over the course of this study. Lukaski et al. has suggested that tissue mobilization of Zn is impaired when low dietary Zn intake compromises circulating Zn status (73).

CONCLUSION

Prolonged, intense aerobic exercise (> 1 hour at 80% of AT) produced a significant change in plasma and erythrocyte concentrations of Zn and Cu over a five day post-exercise time period. A highly significant decline occurred in erythrocyte content of

Zn and Cu from PRE to PE-5D. Increases from PRE to PE and decreases from PE to PE-2H were highly significant for both plasma-Zn and plasma-Cu. A shift of Zn from erythrocytes to plasma appeared to take place from PRE to PE. Copper also appeared to shift from erythrocytes to plasma during the exercise period and then shift back from plasma to erythrocytes between PE-2H and PE-1D. Urine excretions of Zn and Cu over the post-exercise time period were not significantly different from those at PRE.

Subjects, as a group, were able to maintain plasma concentrations of Zn and Cu after participating in acute, intense aerobic exercise while consuming unsupplemented self-selected diets. However, hypozincemia was evident in 26% the cyclists. The subject group was not able to maintain erythrocyte concentrations of Zn and Cu under these exercise and dietary conditions.

The data do not appear to support that Zn and Cu status can be maintained in both plasma and erythrocytes when subjects participate in acute, intense and prolonged aerobic exercise while consuming self-selected unsupplemented diets. The sample size of the present study was small, therefore, it would be interesting to determine if these results were duplicable with a larger athletic population. These competitive cyclists were in the early weeks of their racing season and did not cease training during the protocol; rather, any aerobic training was performed at < 60% of AT. Based on the results of the present study, normal strenuous aerobic training on a daily basis would be expected to progressively compromise circulating Zn and Cu status, predominantly in erythrocyte concentrations. Consequently, over the training and racing season, these cyclists may be

susceptible to decrements of antioxidant function, energy metabolism and endurance. A primary recommendation would be to improve the selection of foods high in Zn (see Table 1) and Cu (see Table 2) to enrich the diet quality and consistently achieve RDA and ESADDI levels, respectively. Judicious supplementation of dietary Zn and Cu may also be a viable option for these cyclists to maintain Zn and Cu status in erythrocytes, as well as plasma.

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APPENDIX A

USCF RACING CATEGORY DEFINITIONS

USCF RACING CATEGORIES

United States Cycling Federation
One Olympic Plaza
Colorado Springs, CO 80909-5775
719/578-4949

CATEGORY: ROAD - upgrade requirements:

5 - Novice: 10 mass start qualifying races
races: road = 15 miles
criterium = 10 miles/45 minutes
minimum field size = 10

4 - Amateur: 15 points/12 months: 25 qualifying races or 5 top ten finishes
races: road = 25 miles
criterium = 20 miles/60 minutes
minimum field size = 30

3 - 30 points/12 months: 25 qualifying races or 5 top ten finishes
races: road = 50 miles
criterium = 20 miles/60 minutes
minimum field size = 50

2 - Semi-professional:
30 points/12 months: 25 qualifying races or 5 top ten finishes
races: road = 80
criterium = 35 miles/90 minutes
field size = 60

1 - Professional Cyclist

POINT SYSTEM:

For categories 3-4-5 for road or criterium:
1st = 7, 2nd = 5, 3rd = 4, 4th = 3, 5th = 2, 6th = 1

For categories 3 for road:
1st = 10, 2nd = 7, 3rd = 5, 4th = 3, 5th = 2, 6th = 1
- for criterium:
1st = 7, 2nd = 5, 3rd = 4, 4th = 3, 5th = 2, 6th = 1

For categories 2 for road:

1st = 10, 2nd = 7, 3rd =5, 4th = 3, 5th = 2, 6th = 1

- for criterium:

1st = 7, 2nd = 5, 3rd = 4, 4th = 3, 5th = 2, 6th = 1

- plus Stage Racing requirements

CATEGORY: OFF-ROAD

E - Expert

S - Sport

APPENDIX B

INFORMED LETTER OF CONSENT

Informed Consent for "The Effects of an Acute Bout of Strenuous Prolonged Aerobic Exercise on Plasma, Erythrocyte, Urinary, and Dietary Values for Selected Trace Minerals"

You are invited to participate in a study to evaluate the effects of an acute, strenuous bout of exercise on trace mineral metabolism. We hope to learn if mineral levels are maintained within normal value ranges for trained endurance cyclists following an acute bout of exercise. You were selected as a possible participant because of your exercise history.

If you decide to participate, you will be asked to do the following:

1. Not to take any form of dietary mineral supplement for one month prior to the initiation of this study, and during the one-week time frame of this study.
2. Keep daily food records for the duration of this study.
3. Allow a trained phlebotomist to collect blood before, immediately after completion of the exercise protocol, two hours post-exercise, one, three, and five days post-exercise. A total of 30 ml (approximately 2 tablespoons) of blood will be drawn from a forearm vein each time blood is drawn.
4. Keep 24-hour urine collections for the 24-hours prior to the test ride, the day of the ride, and on days 3 and 5 post-exercise.
5. Not to participate in any strenuous sporting event(s) that may alter the test results of this study for the five days following the exercise protocol.

This study presents only minimal risks for each participant. There is a remote chance of a low grade local infection with venous blood collection. This type of infection may show some redness and possible inflammation, but is reversed by the body's immune system. The use of aseptic techniques reduces the possibility of this type of local infection. The benefits to the subjects will be an analysis of their dietary and mineral status over the duration of the study. All participants will receive an analysis of the results obtained from their participation in this study.

It is understood that if you are giving your consent to be a participant in this study that you do not have a current or past condition of the following: (a) coronary heart disease, (b) rheumatoid disease(s), (c) type I or type II diabetes, (d) any vascular disease, (e) anemia, and (f) currently taking a prescription drug that affects heart rate (e.g. beta blockers).

Subject's Initials _____

Any information obtained in connection with you will remain confidential and be disclosed only with your permission. Only data averaged for several subjects will be disclosed in scientific publications. Your decision whether or not to participate will not prejudice your future relationship with Texas Woman's University or Texas Christian University. If you decide to participate, you are free to withdraw as a subject at any time during this study. Should injury result during participation in this study, you will be required to seek you own medical care. Upon completion of this study, all participants will receive a copy of their participation in this research investigation.

If you have any questions before, during or after the study, Kim Gramenz, RD, Principle Investigator (214-221-7193), Ginger Schirmer, PhD, RD, Research Director (817-921-7309), Dan Southard, PhD, Chair of Committee of Safeguards and Human Subjects (817-921-7665), or Jan Fox, Director of Office Sponsored Projects (817-921-7516), and we will be happy to answer them. You will be given a copy of this form to keep. You are making an informed decision whether or not to participate in this study. Your signed signature indicates that you have decided to participate having read the information provided above and having been informed about procedures.

Date Time	Subject's Signature
Witness	Investigator's Signature

APPENDIX C

HEALTH STATUS QUESTIONNAIRE

Health Status Questionnaire

Instructions: Complete each question accurately. All information provided is confidential.

Part I. Information about the individual:

1. Name: _____
2. Mailing Address: _____
3. Home Phone Number: ( ) _____
4. Work Phone Number: ( ) _____
5. Personal Physician: _____
6. Address and Phone Number of Personal Physician:
   _____
   _____
   _____
7. Gender: M or F
8. Age: _____
9. Height: _____
10. Weight: _____
11. Number of hours worked per week: less than 20 20-40 41-60 over 60
12. More than 25% of time spent on the job (circle all that apply):
    Sitting at desk Lifting or carrying loads Standing Walking Driving

Part II. Medical History

13. Circle any relation who died of a heart attack before age 50:
    Father Mother Brother Sister Grandparent

14. Date of:
    a. Last medical physical exam (month/year): _____
    b. Last physical fitness test (month/year): _____

15. Have you had any surgeries? If so, please list surgeries and year they were performed: _____
    _____

16. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

alcoholism	diabetes	kidney problem
anemia, sickle cell	emphysema	mental illness
anemia, other	epilepsy	neck strain
asthma	eye problems	obesity
back strain	gout	phlebitis
bronchitis, chronic	hearing loss	rheumatoid arthritis
cancer	heart problems	stroke
cirrhosis, liver	high blood pressure	thyroid problem
concussion	hypoglycemia	ulcer
infectious mononucleosis	congenital defect	hyperlipidemia
other:		

17. Please list all medications currently being taken or taken during the past year:
    _____
    _____

18. Any of these health symptoms that occurs frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:
    5 = Very Often
    4 = Fairly Often
    3 = Sometimes
    2 = Infrequently
    1 = Practically Never
    N/A = Not Applicable

Cough up blood	1 2 3 4 5 N/A
Abdominal pain	1 2 3 4 5 N/A
Low back pain	1 2 3 4 5 N/A
Leg pain	1 2 3 4 5 N/A
Arm or shoulder pain	1 2 3 4 5 N/A

Chest pain	1 2 3 4 5 N/A
Swollen joints	1 2 3 4 5 N/A
Feel faint	1 2 3 4 5 N/A
Dizziness	1 2 3 4 5 N/A
Breathless w/ slight exertion	1 2 3 4 5 N/A

Part III. Exercise History

19. How many days per week do you exercise? _____

20. What type of exercise modes do you predominately perform?

    _____

    _____

21. How many years have you been exercising regularly? _____

22. How many hours per week do you normally participate in physical activity? _____

APPENDIX D

CONCONI TEST FOR ANAEROBIC THRESHOLD

Conconi Test for Anaerobic Threshold

Set-up for stand alone mode requires initial calibration of the CompuTrainer

Testing Steps:

1. Warm up 10-15 minutes gradually raising the intensity.

2. Select a gear that would be appropriate to time trial up a gradual hill, such as 53 x 15 or 16. Write this gear down for future reference. You will stay in this gear the entire test and any subsequent retests.

3. Start the test at 18 mph in your selected gear with watts set for 50. Every minute your assistant will increase the watts by 20 by pressing the "+" key twice. You will stay in the same gear and attempt to maintain a constant 18 mph. Do this until you can no longer maintain 18 mph. Stay seated - do not get out of the saddle throughout the test. At the end of every minute your assistant records your heart rate (HR), watts, and perceived exertion value (PEV). Use the following PEV guide:

7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

4. Your assistant should also listen to your breathing to detect when it first becomes labored. Typically, AT HR occurs at the same time as the ventilatory threshold (VT). The assistant should mark the heart rate at

this point on the data-keeping sheet as a reference point. The data will look like this:

Watts	HR	PEV	
50	110	9	
70	118	11	
90	125	12	
110	135	13	
130	142	14	
150	147	15	
170	153	17	"VT"
190	156	19	

5. Create an "XY" graph with the vertical coordinate representing HR and the horizontal coordinate representing watts.

6. Plot the data points from the test onto the chart and connect them. The point at which the line deflects or bends sharply is assumed to be AT HR. Confirm this by comparing it with the VT HR and PEV of 16 or 17. Another way of confirming it is that most riders can usually only achieve three to five data points beyond their AT. Be aware that many people don't have a deflection point. If you are in this group, assume that VT pulse matched by 16 or 17 PEV is your AT HR.

APPENDIX E

DAILY FOOD DIARY

Name: ______________________________

THE EFFECTS OF AN ACUTE BOUT OF STRENUOUS AEROBIC EXERCISE ON PLASMA, ERYTHROCYTE, URINARY AND DIETARY VALUES FOR SELECTED TRACE MINERALS

Daily Food Diary

Day 1 2 3 4 5 6 or 7

Food or Beverage Consumed	Quantity (in standard measures)
Example: Spaghetti	4 c noodles
w/ Healthy Choice meat sauce	1 1/2 c sauce
Garlic bread w/ margarine	2 (1") slices w/ 2 tsp margarine
Steamed broccoli	2 c
Water	20 oz
Power Bar - chocolate	1

APPENDIX F

DIETARY DATA

Energy Intake Analysis of Study Subjects (expressed in kcal)

	Day						
	1	2	3	4	5	6	Mean
Subject 1	4,007	2,799	2,958	3,656	2,915	3,381	3,286
Subject 2	4,119	5,131	3,530	4,127	4,063	5,281	4,375
Subject 3	3,720	3,974	2,947	2,996	2,823	2,298	3,126
Subject 4	1,873	3,588	2,022	2,348	3,439	2,499	2,628
Subject 5	3,761	5,456	3,720	4,107	3,747	2,337	3,855
Subject 6	6,076	2,920	6,690	5,204	2,654	3,103	4,441
Subject 8	4,349	4,070	3,860	2,448	2,806	2,166	3,283
Subject 9	4,331	3,561	2,454	1,995	2,016	3,199	2,926
Subject 10	1,037	2,307	3,453	2,111	2,248	2,897	2,342
Subject 11	3,252	3,011	3,463	3,185	3,124	2,594	3,105
Subject 12	3,098	3,952	1,922	4,208	3,617	2,608	3,234
Subject 13	2,541	3,278	2,887	3,514	2,765	2,873	2,976
Subject 14	3,606	3,499	2,883	1,693	3,366	3,442	3,082
Subject 15	1,925	1,881	3,179	1,691	2,392	2,152	2,203
Subject 16	2,462	4,056	4,151	4,539	3,130	3,645	3,664
Subject 17	2,904	3,592	3,411	3,322	1,830		3,012
Subject 18	5,616	3,900	4,476	3,582	6,111	6,854	5,090
Subject 19	3,750	2,503	3,309	2,985	3,037	2,872	3,076

Descriptive Statistics

	1	*2*	*3*	*4*	*5*	*6*	*Average*
Mean	3,468	3,527	3,406	3,206	3,116	3,188	3,317
Standard Error	298	213	248	239	224	292	173
Median	3,663	3,575	3,360	3,254	2,976	2,873	3,116
Standard Deviation	1,263	903	1,053	1,016	951	1,205	734
Range	5,039	3,575	4,768	3,513	4,281	4,702	2,887
Minimum	1,037	1,881	1,922	1,691	1,830	2,152	2,203
Maximum	6,076	5,456	6,690	5,204	6,111	6,854	5,090
Count	18	18	18	18	18	17	18
Confidence Level(95%)	628	449	524	505	473	619	365

Dietary Carbohydrate Intake Analysis of Study Subjects (expressed in grams)

	DAY						
	1	2	3	4	5	6	Average
Subject 1	637	423	521	560	546	552	540
Subject 2	569	463	391	567	643	823	576
Subject 3	423	343	355	292	472	308	366
Subject 4	227	460	227	232	504	403	342
Subject 5	579	886	501	638	692	218	586
Subject 6	853	451	812	784	507	460	645
Subject 8	710	634	550	352	340	280	478
Subject 9	670	534	384	268	304	301	410
Subject 10	173	307	536	277	360	506	360
Subject 11	447	392	467	338	406	338	398
Subject 12	412	425	212	547	476	283	393
Subject 13	349	424	423	432	344	349	387
Subject 14	355	487	457	261	416	405	397
Subject 15	243	330	606	281	443	373	379
Subject 16	451	540	706	718	340	605	560
Subject 17	477	438	455	563	341		455
Subject 18	898	702	684	700	961	1083	838
Subject 19	345	306	481	416	343	387	380

Descriptive Statistics

	1	*2*	*3*	*4*	*5*	*6*	*Average*
Mean	490	475	487	457	469	451	472
Standard Error	48	35	36	42	39	53	31
Median	449	445	474	424	430	387	404
Standard Deviation	206	147	154	180	164	219	130
Range	725	580	600	552	657	865	496
Minimum	173	306	212	232	304	218	342
Maximum	898	886	812	784	961	1,083	838
Count	18	18	18	18	18	17	18
Confidence Level (95%)	102	73	76	90	82	113	65

Dietary Protein Intake Analysis of Study Subjects (expressed in grams)

	Day							Percent
	1	2	3	4	5	6	Average	of RDA
Subject 1	147	117	98	102	112	100	113	194%
Subject 2	112	167	121	85	108	213	134	232%
Subject 3	157	207	153	133	110	151	152	241%
Subject 4	92	122	101	116	118	102	109	187%
Subject 5	137	184	180	181	155	67	151	239%
Subject 6	212	131	211	175	102	127	160	253%
Subject 8	103	125	186	94	190	115	136	234%
Subject 9	140	162	95	92	78	139	118	187%
Subject 10	28	124	117	114	87	83	92	159%
Subject 11	113	101	92	100	177	129	119	205%
Subject 12	109	110	92	151	116	85	111	191%
Subject 13	92	132	75	191	79	116	114	181%
Subject 14	295	121	140	150	171	192	178	283%
Subject 15	78	69	111	69	71	95	82	130%
Subject 16	78	188	189	179	142	109	148	234%
Subject 17	65	172	145	108	40		106	183%
Subject 18	234	131	188	122	276	270	204	323%
Subject 19	159	85	118	94	86	100	107	170%
Average								213%

Descriptive Statistics

	1	*2*	*3*	*4*	*5*	*6*	*Average*
Mean	131	136	134	125	123	129	129
Standard Error	15	9	10	9	13	13	7
Median	113	128	120	115	111	115	118
Standard Deviatio	65	37	42	37	55	52	31
Range	267	138	136	122	236	203	121
Minimum	28	69	75	69	40	67	82
Maximum	295	207	211	191	276	270	204
Count	18	18	18	18	18	17	18
Confidence Level(95%)	32	18	21	19	27	27	15

Dietary Zinc Intake Analysis of Study Subjects (expressed in mg)

	DAY							Percent
	1	2	3	4	5	6	Average	of RDA
Subject 1	20.56	10.03	7.48	12.45	10.12	13.79	12.41	82.70%
Subject 2	16.30	21.77	9.60	2.65	4.35	30.63	14.22	94.78%
Subject 3	17.46	29.75	13.61	18.25	14.53	13.80	17.90	119.33%
Subject 4	9.36	14.18	4.40	12.59	7.70	6.03	9.04	60.29%
Subject 5	15.49	20.79	12.73	17.95	9.57	10.86	14.57	97.10%
Subject 6	29.00	12.43	29.19	30.38	11.69	16.13	21.47	143.13%
Subject 8	8.20	14.85	21.32	5.59	15.76	7.54	12.21	81.40%
Subject 9	16.61	9.35	8.56	6.09	2.09	11.70	9.07	60.44%
Subject 10	3.45	14.83	12.07	9.09	9.64	10.05	9.86	65.70%
Subject 11	14.65	10.20	11.48	11.23	16.00	6.08	11.61	77.38%
Subject 12	13.07	10.49	12.81	17.28	7.96	11.11	12.12	80.80%
Subject 13	5.90	21.00	10.89	18.77	6.83	2.85	11.04	73.60%
Subject 14	31.27	7.08	13.20	9.96	17.71	9.35	14.76	98.41%
Subject 15	12.84	8.95	12.02	6.88	9.97	6.76	9.57	63.80%
Subject 16	14.31	24.91	29.16	21.71	20.67	14.03	20.80	138.66%
Subject 17	7.96	15.53	17.55	7.21	2.75		10.20	68.00%
Subject 18	27.44	15.03	27.03	14.09	24.05	37.01	24.11	160.72%
Subject 19	21.05	10.29	13.19	7.15	8.24	13.69	12.27	81.79%
Average								91.56%

Descriptive Statistics

	1	*2*	*3*	*4*	*5*	*6*	*Average*
Mean	15.83	15.08	14.79	12.74	11.09	13.02	13.73
Standard Error	1.84	1.47	1.71	1.64	1.41	2.10	1.06
Median	15.07	14.51	12.77	11.84	9.81	11.11	12.24
Standard Deviation	7.80	6.23	7.26	6.96	6.00	8.66	4.51
Range	27.82	22.67	24.79	27.73	21.96	34.16	15.07
Minimum	3.45	7.08	4.40	2.65	2.09	2.85	9.04
Maximum	31.27	29.75	29.19	30.38	24.05	37.01	24.11
Count	18	18	18	18	18	17	18
Confidence Level(95%)	3.88	3.10	3.61	3.46	2.98	4.45	2.24

Dietary Copper Intake Analysis of Study Subjects (expressed in mg)

	DAY							Percent of ESADDI	
	1	2	3	4	5	6	Average	Lower	Upper
Subject 1	0.92	1.34	0.99	1.23	1.25	1.75	1.25	83.11%	41.56%
Subject 2	2.36	0.87	1.54	0.36	0.75	0.56	1.07	71.56%	35.78%
Subject 3	2.28	2.40	1.92	2.18	3.70	1.61	2.35	156.56%	78.28%
Subject 4	2.10	2.56	0.27	1.37	1.47	0.81	1.43	95.33%	47.67%
Subject 5	2.30	3.26	1.34	2.36	1.57	2.54	2.23	148.56%	74.28%
Subject 6	3.71	1.85	1.81	1.92	2.24	2.56	2.35	156.56%	78.28%
Subject 8	1.50	2.89	3.04	0.73	2.38	1.18	1.95	130.22%	65.11%
Subject 9	1.11	1.78	1.06	1.16	0.51	2.01	1.27	84.78%	42.39%
Subject 10	0.26	1.01	2.16	1.42	1.80	1.96	1.44	95.67%	47.83%
Subject 11	2.27	1.08	1.79	1.05	1.70	0.54	1.41	93.67%	46.83%
Subject 12	2.30	2.36	1.32	1.36	1.19	1.06	1.60	106.56%	53.28%
Subject 13	0.85	0.64	1.39	1.52	0.91	0.68	1.00	66.56%	33.28%
Subject 14	1.25	1.83	1.18	1.65	1.53	1.24	1.45	96.44%	48.22%
Subject 15	2.53	1.40	2.38	1.04	1.69	1.26	1.72	114.44%	57.22%
Subject 16	1.86	2.33	3.01	4.12	1.99	1.67	2.50	166.44%	83.22%
Subject 17	1.36	0.62	0.92	0.99	0.75		0.93	61.87%	30.93%
Subject 18	2.51	2.31	1.90	2.28	4.33	2.05	2.56	170.89%	85.44%
Subject 19	1.14	1.55	2.08	1.29	1.56	2.05	1.61	107.44%	53.72%
Average								111.48%	55.74%

Descriptive Statistics

	1	*2*	*3*	*4*	*5*	*6*	*Average*
Mean	1.81	1.78	1.67	1.56	1.74	1.50	1.67
Standard Error	0.19	0.18	0.17	0.20	0.23	0.16	0.12
Median	1.98	1.81	1.67	1.37	1.57	1.61	1.52
Standard Deviation	0.83	0.78	0.71	0.83	0.98	0.65	0.53
Range	3.45	2.64	2.77	3.76	3.82	2.02	1.64
Minimum	0.26	0.62	0.27	0.36	0.51	0.54	0.93
Maximum	3.71	3.26	3.04	4.12	4.33	2.56	2.56
Count	18	18	18	18	18	17	18
Confidence Level (95%)	0.41	0.39	0.35	0.41	0.48	0.33	0.26

APPENDIX G

DETERMINATION OF

ZINC

Determination of Zinc (Zn)

Reagents and Procedure

A. Preparation of Standards and Solutions for plasma-Zn, erythrocyte-Zn and urine-Zn:

1. Zinc Reference Standard (1 ml = 1000 μg Zn [1000 ppm]) (Ricca Chemical Co.)
2. For the Standard Curve the following concentrations (μg/ml) were prepared: blank, 0.1, 0.5, 1.0, 1.5, and 2.0
3. For 0.1 μg/ml: In 100 ml flask, 5 ml glycerol was added and 10 μl zinc standard was pipetted with Eppendorf Digital Pipette; the flask was brought to 100 ml volume with DIW.
4. Step # 3 was repeated to prepare the next four concentrations using 50, 100, 150, and 200 μl zinc standard, respectively.
5. 5% Glycerol Solution - In 1L flask, 50 ml glycerol was added and brought to 1L volume with DIW.

B. Preparation of Atomic Absorption Spectrophotometer (AAS):

1. Air/acetylene flame was set according to standard procedures by Varian (139).
2. Wavelength was 213.9 nm and slit setting was 4 (0.7 nm).
3. Aspiration rate was 5 ml/minute.
4. Zinc hollow cathode lamp was selected.
5. Standard curve was established using prepared standards.

C. Preparation of Samples:

1. Into sterile, trace-mineral free polypropylene 6 ml test tubes (Becton Dickinson), the following were added:
   a. Plasma samples - 500 μl plasma + 2 ml DIW for a 1:4 ratio
   b. Erythrocyte samples - 50 μl erythrocytes 1:1 in DIW + 2 ml DIW for a 1:80 ratio
   c. Urine samples - 1ml urine + 1 ml of 5% Glycerol Solution for a 1:1 ratio
2. With AAS set for zinc, each sample was aspirated and analyzed in triplicate readings (μg/ml concentration).
3. Samples were prepared in duplicate.
4. Accuracy and precision were evaluated by analyzing a known standard concentration after every two samples.
5. Values with $\leq$ 5.0 % relative standard deviation (%RSD) for the triplicate readings were acceptable.

D. Calculations for plasma-Zn, erythrocyte-Zn and urine-Zn:

1. Plasma-Zn
   a. AAS sample reading in μg/ml x 5 (dilution factor) = sample plasma-Zn (μg/ml)
   b. Sample plasma-Zn μg/ml x 100 = sample plasma-Zn μg/dl
   c. Sample plasma-Zn μg/dl x 0.1530 (SI conversion factor) = sample plasma-Zn concentration μmol/L
2. Erythrocyte-Zn
   a. AAS sample reading in μg/ml x 81 (dilution factor) = sample erythrocyte-Zn (μg/ml)
   b. Sample erythrocyte-Zn μg/ml x 100 = sample erythrocyte-Zn μg/dl
   c. Sample erythrocyte-Zn μg/dl x 0.1530 (SI conversion factor) = sample erythrocyte-Zn concentration μmol/L
3. Urine-Zn
   a. AAS sample reading in μg/ml x 2 (dilution factor) = sample urine-Zn (μg/ml)
   b. Sample urine-Zn μg/ml x urine 24 hour volume in ml = sample urine-Zn μg/24 hr
   c. Sample urine-Zn μg/24 hour x 0.01530 (SI conversion factor) = sample urine-Zn excretion μmol/day

APPENDIX H

DETERMINATION OF

COPPER

Determination of Copper (Cu)

Reagents and Procedure

A. Preparation of Standards and Solutions for plasma-Cu and erythrocyte-Cu:

1. Copper Reference Standard (1 ml = 1000 µg Zn [1000 ppm]) (Ricca Chemical Co.)
2. For the Standard Curve the following concentrations (µg/ml) were prepared: blank, 0.03, 0.05, 0.08 and 0.1
3. For 0.03 µg/ml: In 100 ml flask, 5 ml glycerol was added and 3 µl Cu standard was pipetted with Eppendorf Digital Pipette; the flask was brought to 100 ml volume with DIW.
4. Step # 3 was repeated to prepare the next three concentrations using 5, 8 and 10 µl Cu standard, respectively.

B. Preparation of Atomic Absorption Spectrophotometer (AAS):

1. Air/acetylene flame was set according to standard procedures by Varian (139).
2. Wavelength was 324.7 nm and slit setting was 4 (0.7 nm).
3. Aspiration rate was 5 ml/minute.
4. Copper hollow cathode lamp was selected.
5. Standard curve was established using prepared standards.

C. Preparation of plasma-Cu and erythrocyte-Cu Samples:

1. Into sterile, trace-mineral free polypropylene 6 ml test tubes (Becton Dickinson), the following were added:
   a. Plasma samples - 500 µl plasma + 4.0 ml DIW for a 1:8 ratio
   b. Erythrocyte samples - 500 µl erythrocytes 1:1 in DIW + 3 ml DIW for a 1:14 ratio
2. With AAS set for Cu, each sample was aspirated and analyzed in triplicate readings (µg/ml concentration).
3. Samples were prepared in duplicate.
4. Accuracy and precision were evaluated by analyzing a known standard concentration after every two samples.
5. Values with $\leq$ 8.0 % relative standard deviation for the triplicate readings were acceptable.

D. Calculations for plasma-Cu and erythrocyte-Cu:

1. Plasma-Cu
   a. AAS sample reading in μg/ml x 9 (dilution factor) = sample plasma-Cu (μg/ml)
   b. Sample plasma-Cu μg/ml x 100 = sample plasma-Cu μg/dl
   c. Sample plasma-Cu μg/dl x 0.1574 (SI conversion factor) = sample plasma-Cu concentration in μmol/L
2. Erythrocyte-Cu
   a. AAS sample reading in μg/ml x 15 (dilution factor) = sample erythrocyte-Cu (μg/ml)
   b. Sample erythrocyte-Cu μg/ml x 100 = sample erythrocyte-Cu μg/dl
   c. Sample erythrocyte-Cu μg/dl x 0.1574 (SI conversion factor) = sample erythrocyte-Cu concentration in μmol/L

E. Preparation of Graphite Furnace AAS (GFAAS) and samples:

1. Instrument was programmed according to standard operating procedures by Varian for autosampler without background correction (140).
2. Wavelength was set at 324.8.
3. Standards (in ng/ml) for sampler parameters were: blank, 2.0, 5.0, 8.0 and 10.0.
4. A 1% nitric acid solution was made by adding 1 ml nitric acid to a 100 ml flask and filling to 100 ml volume with DIW.
5. Sampler parameter volumes (ng/ml) were determined; solution is Cu referene standard and blank is 1% nitric acid:

	solution	blank
blank	0	20
standard 1 is 2.0	4	16
standard 2 is 5.0	10	10
standard 3 is 8.0	16	4
standard 4 is 10.0	20	0
sample	10	10

6. Urine samples were prepared by pipetting 100 μl + 1.5 ml 1% nitric acid into GFAAS sterile, polypropylene autosampler cups.
7. Standards and samples were analyzed in triplicate.
8. Accuracy and precision were evaluated by analyzing a standard sample after every ten samples.

F. Calculation of urine-Cu:

a. GFAAS sample concentration in ng/ml x 32 (*dilution factor)
= sample urine-Cu(ng/ml)

b. Sample urine-Cu ng/ml ÷ 1000 = sample urine-Cu μg/ml

c. Sample urine-Cu μg/ml x urine 24 hour volume in ml
= sample urine-Cu μg/24 hour

d. Sample urine-Cu μg/24 hour x 0.01574 (SI conversion factor)
= sample urine-Cu excretion μmol/day

* Dilution factor of 1:15 with 1% nitric acid + 1:1 autosampler dilution = 1:31 ratio

APPENDIX I

BIOCHEMICAL DATA

Plasma Zinc Values of Study Subjects

	PLASMA 11.50-18.50 umol/L					
Subject	A	B	C	D	E	F
1	11.55	13.77		14.54	16.22	11.48
2	13.85	15.30	13.01	12.24	13.77	12.24
3	13.77	15.30	12.24	13.77	14.54	14.54
4	14.15	16.07	12.24	17.60	15.30	13.01
5	14.23	16.07	13.77	10.71	12.78	13.77
6	13.01	13.77	12.24	13.01	13.77	11.09
7	12.24	15.30	12.24	10.71	12.24	11.48
8	15.30		12.24	12.24	18.51	17.82
9	13.01	13.77	12.24	13.01	13.01	14.23
10	11.78	13.77	12.24	11.48	13.01	13.01
11	12.24	14.92	12.39	13.01	12.24	12.24
12	10.71	13.01	10.63		10.63	10.71
13	10.71	13.01	12.55	11.48	10.71	14.99
14	13.01	16.83	14.54	15.30	13.77	16.45
15	11.93	14.46	10.63	12.24	13.01	16.07
16	12.93	15.30	14.54	13.01	13.77	12.24
17	13.92	16.07	10.71	12.55	13.01	13.92
18	11.55	13.01	14.54	12.24	11.48	11.48
19	13.77	16.07	12.24	13.01	13.77	16.07
Average	12.82	14.77	12.51	12.90	13.45	13.52

Descriptive Statistics

	A	*B*	*C*	*D*	*E*	*F*
Mean	12.82	14.77	12.51	12.90	13.45	13.52
Standard Error	0.29	0.29	0.29	0.39	0.43	0.47
Median	13.01	15.11	12.24	12.78	13.01	13.01
Standard Deviation	1.26	1.23	1.22	1.66	1.85	2.05
Range	4.59	3.82	3.91	6.89	7.88	7.11
Minimum	10.71	13.01	10.63	10.71	10.63	10.71
Maximum	15.30	16.83	14.54	17.60	18.51	17.82
Count	19	18	18	18	19	19
Confidence Level(95%)	0.61	0.61	0.60	0.83	0.89	0.99

Erythrocyte Zinc Values of Study Subjects

	RBC 135-245 umol/L					
Subject	A	B	C	D	E	F
1	169.4	213.3		213.3	175.6	194.5
2	175.0	175.0	169.4	156.8	169.4	169.4
3	187.0	168.8	156.8	168.8	156.8	144.9
4	184.4	172.6	169.5	178.1	184.4	178.1
5	179.9	153.8	169.5	178.1	190.6	190.6
6	204.6	197.7	207.0	213.3	232.1	232.1
7	211.4	180.7	180.7	193.2	193.2	193.2
8	195.1		195.1	183.1	177.5	195.1
9	156.8	156.8	150.6	144.3	138.0	138.0
10	169.4	163.1	163.1	169.4	163.1	156.8
11	200.1	193.9	187.0	160.0	160.0	173.8
12	205.2	175.0	181.9		163.1	194.5
13	194.5	169.4	150.6	144.3	169.4	175.6
14	188.2	163.1	144.3	156.8	156.8	175.6
15	188.2	174.4	130.5	149.3	164.3	168.8
16	183.1	178.9	156.8	177.5	167.5	141.2
17	188.2	188.2	181.9	169.4	163.1	175.6
18	205.2	179.5	173.2	173.2	166.3	166.3
19	137.4	118.0	118.0	130.5	124.2	150.6
Average	185.4	173.5	165.9	170.0	169.2	174.5

Descriptive Statistics

	A	*B*	*C*	*D*	*E*	*F*
Mean	185.43	173.46	165.88	169.97	169.23	174.46
Standard Error	4.20	4.76	5.26	5.24	5.09	5.27
Median	188.20	174.70	169.45	169.40	166.30	175.60
Standard Deviation	18.30	20.19	22.32	22.25	22.20	22.97
Range	74.00	95.30	89.00	82.80	107.90	94.10
Minimum	137.40	118.00	118.00	130.50	124.20	138.00
Maximum	211.40	213.30	207.00	213.30	232.10	232.10
Count	19.00	18.00	18.00	18.00	19.00	19.00
Confidence Level (95%)	8.82	10.04	11.10	11.07	10.70	11.07

Urine Zinc Values of Study Subjects

	URINE 2.30-18.30 umol/d				
Subject	PRE	EX	PE-1	PE-2	PE-4
1		6.85		4.31	7.44
2	18.97	14.63		11.67	19.09
3	11.93	9.67		10.40	6.79
4	10.33	9.41		6.72	11.29
5	11.34	9.33		9.73	7.86
6	14.21	14.27	10.39	10.63	7.60
7	12.74	19.37	15.58	13.97	18.65
8	8.20	1.47		18.15	9.81
9	9.07	7.05		7.27	5.97
10	11.12	12.67		12.39	12.53
11	17.46	6.76		8.69	16.94
12	6.17	8.46	19.06	14.86	8.48
13	7.83	11.44		11.14	8.09
14	12.65	12.10		16.43	14.66
15	11.54	8.25	6.38	7.51	7.39
16	10.66	10.17	7.30	6.90	7.65
17	8.51	8.35	10.30	10.99	8.90
18	15.12	11.37	6.62	11.05	17.98
19	8.66	13.40		15.38	18.50
Average	11.47	10.26	10.80	10.96	11.35

Descriptive Statistics

	PRE	EX	PE-1	PE-2	PE-4
Mean	11.47	10.26	10.80	10.96	11.35
Standard Error	0.79	0.88	1.83	0.84	1.08
Median	11.23	9.67	10.30	10.99	8.90
Standard Deviation	3.37	3.83	4.84	3.65	4.71
Range	12.80	17.90	12.68	13.84	13.12
Minimum	6.17	1.47	6.38	4.31	5.97
Maximum	18.97	19.37	19.06	18.15	19.09
Count	18.00	19.00	7.00	19.00	19.00
Confidence Level(95%)	1.67	1.85	4.48	1.76	2.27

Plasma Copper Values of Study Subjects

	PLASMA 11.50-18.50 umol/L					
Subject	PRE	PE	PE-2H	PE-1D	PE-3D	PE-5D
1	22.20	23.23		22.67	18.20	17.20
2	17.20	20.24	19.23	15.18	15.35	22.67
3	18.89	22.38	21.25	21.25	17.94	20.86
4	16.53	21.25	21.25	19.83	19.18	20.86
5	17.94	21.25	19.83	15.58	21.25	21.25
6	12.59	12.59	8.66	9.44	13.38	11.02
7	15.94	14.17	14.17	15.05	14.17	15.05
8	16.13		16.13	17.31	17.31	17.31
9	17.71	17.71	18.84	13.38	15.74	15.43
10	20.23	21.17	22.58	18.33	18.61	21.17
11	18.42	21.25	19.83	19.83	18.42	19.83
12	14.87	16.05	13.46		14.87	14.64
13	18.42	18.42	15.58	17.00	15.58	15.58
14	17.71	18.84	17.71	16.57	16.57	15.30
15	15.05	16.82	15.94	16.82	14.17	15.94
16	17.00	17.00	15.58	15.58	17.00	17.00
17	16.68	18.10	11.81	14.04	16.13	15.43
18	16.53	17.79	14.95	14.17	17.94	17.94
19	17.00	21.25	19.83	15.58	15.58	17.00
Average	17.21	18.86	17.04	16.53	16.70	17.45

Descriptive Statistics

	PRE	PE	PE-2H	PE-1D	PE-3D	PE-5D
Mean	17.21	18.86	17.04	16.53	16.70	17.45
Standard Error	0.47	0.68	0.86	0.73	0.46	0.68
Median	17.00	18.63	16.92	16.08	16.57	17.00
Standard Deviation	2.06	2.89	3.66	3.11	2.00	2.97
Range	9.61	10.64	13.92	13.23	7.87	11.65
Minimum	12.59	12.59	8.66	9.44	13.38	11.02
Maximum	22.20	23.23	22.58	22.67	21.25	22.67
Count	19	18	18	18	19	19
Confidence Level(95%)	0.99	1.44	1.82	1.55	0.96	1.43

Erthrocyte Copper Values of Study Subjects

	RBC 10.38-17.63 umol/L					
Subject #	A	B	C	D	E	F
1	9.92	9.92		11.02	11.02	9.92
2	9.92	9.92	11.02	11.02	9.92	11.02
3	11.02	11.02	11.02	9.92	9.92	9.92
4	14.32	13.22	12.12	12.12	12.12	11.02
5	14.32	14.32	11.02	12.12	11.02	11.02
6	16.53	15.43	13.22	16.53	16.53	16.53
7	14.32	13.22	13.22	13.22	13.22	13.22
8	13.22		13.22	13.22	15.43	13.22
9	15.43	13.22	11.02	11.02	13.22	14.32
10	15.43	13.22	13.22	13.77	14.32	14.32
11	15.43	14.32	15.43	15.43	15.43	14.32
12	14.32	13.22	13.22		13.22	13.22
13	17.63	13.22	13.22	14.32	14.32	14.32
14	15.43	15.43	13.22	14.32	14.32	15.43
15	16.53	15.43	14.32	14.32	15.43	15.43
16	13.22	12.12	12.12	14.32	14.32	14.32
17	15.43	14.32	13.22	13.22	13.22	14.32
18	15.43	13.22	12.12	13.22	14.32	14.32
19	14.32	12.12	12.12	13.22	14.32	14.32
Average	14.32	13.16	12.67	13.13	13.45	13.40

Descriptive Statistics

	A	*B*	*C*	*D*	*E*	*F*
Mean	14.32	13.16	12.67	13.13	13.45	13.40
Standard Error	0.48	0.39	0.29	0.40	0.43	0.44
Median	14.32	13.22	13.22	13.22	14.32	14.32
Standard Deviation	2.11	1.67	1.21	1.69	1.89	1.92
Range	7.71	5.51	4.41	6.61	6.61	6.61
Minimum	9.92	9.92	11.02	9.92	9.92	9.92
Maximum	17.63	15.43	15.43	16.53	16.53	16.53
Count	19	18	18	18	19	19
Confidence Level(95%)	1.02	0.83	0.60	0.84	0.91	0.92

Urine Copper Values of Study Subjects

	URINE <0.630 umol/d				
Subject	PRE	EX	PE-1	PE-2	PE-4
1		0.061		0.027	0.048
2	0.080	0.121		0.056	0.164
3	0.046	0.043		0.030	0.050
4	0.062	0.028		0.037	0.030
5	0.061	0.042		0.047	0.077
6	0.082	0.098	0.118	0.109	0.054
7	0.087	0.099	0.031	0.049	0.032
8	0.021	0.015		0.130	0.091
9	0.023	0.044		0.058	0.053
10	0.078	0.038		0.085	0.060
11	0.095	0.074		0.062	0.184
12	0.026	0.013	0.039	0.040	0.035
13	0.045	0.047		0.064	0.053
14	0.100	0.067		0.082	0.103
15	0.045	0.033	0.050	0.081	0.059
16	0.019	0.043	0.018	0.044	0.054
17	0.052	0.051	0.037	0.085	0.067
18	0.109	0.126	0.087	0.082	0.150
19	0.048	0.062		0.065	0.085
Average	0.060	0.058	0.054	0.065	0.076

Descriptive Statistics

	PRE	EX	PE-1	PE-2	PE-4
Mean	0.0599	0.0582	0.0543	0.0649	0.0763
Standard Error	0.0067	0.0075	0.0134	0.0062	0.0102
Median	0.0565	0.0470	0.0390	0.0620	0.0590
Standard Deviation	0.0284	0.0326	0.0354	0.0269	0.0446
Range	0.0900	0.1130	0.1000	0.1030	0.1540
Minimum	0.0190	0.0130	0.0180	0.0270	0.0300
Maximum	0.1090	0.1260	0.1180	0.1300	0.1840
Count	18	19	7	19	19
Confidence Level(95%)	0.0141	0.0157	0.0328	0.0130	0.0215

APPENDIX J

MATERIALS AND EQUIPMENT LIST

MATERIALS AND EQUIPMENT LIST

1. S/P Alcohol Swabs - saturated with isopropyl alcohol 70% (Baxter Scientifc Products; McGaw Park, IL)

2. Precision-Glide Vacutainer Brand Blood Collection Needles 21g 1/2 (Becton Dickinson; Rutherford, NJ)

3. Vacutainer Brand Evacuated Blood Collection Tubes - 15 ml draw - heparinized (286 USP Units Na+ Heparin) (Becton Dickinson)

4. Disposable Vinyl exam gloves (Ulti-Med)

5. Biohazard Specimen Bag (Lab Guard)

6. Parafilm "M" Laboratory Film (American National Can; Greenwich, CT)

7. Eppendorf Digital Pipettes

8. Polyethylene Sample Container - 3500 ml/1 gallon (Baxter Scientifc Products)

9. Baxter S.P. Clinifuge - Model # 75003538

10 Sartorius Taring Scale (max: 4200 g) - Model # LC 4201S

11. 1.5 ml Conical Bottom w/ Cap Microcentrifuge Tubes (Baxter Scientifc Products)

12. 6 ml Polypropylene Round Bottom Tube w/ Cap (Becton Dickinson)

13. Sodium Chloride - ACS Reagent (Sigma Chemical Co.; St. Louis, MO)

14. Glycerol 'Baker Analyzed' Reagent (J.T. Baker Chemical Co.; Phillipsburg, NJ)

15. Zinc Reference Standard (Ricca Chemical Co.; Arlington, TX)

16. Copper Reference Standard (Ricca Chemical Co.)

17. Breathing Air Cylinder (Rite Weld Supply; Denton, TX)

APPENDIX K

HUMAN SUBJECTS REVIEW COMMITTEE APPROVAL

[redacted]

TEXAS WOMAN'S
UNIVERSITY

August 25, 1995

Kimberly Gramenz
1340 Overlook Drive
Lewisville, Tx 75067

Dear Kimberly Gramenz: [redacted]

Your study entitled "Copper, Selenium, and Zinc Requirements of Endurance Athletes" has been reviewed by a committee of the Human Subjects Review Committee and appears to meet our requirements in regard to protection of individuals' rights.

Be reminded that both the University and the Department of Health and Human Services (HHS) regulations typically require that signatures indicating informed consent be obtained from all human subjects in your study. These are to be filed with the Human Subjects Review Committee. Any exception to this requirement is noted below. Furthermore, according to HHS regulations, another review by the Committee is required if your project changes.

Special provisions pertaining to your study are noted below:

X The filing of signatures of subjects with the Human Subjects Review Committee is not required.

X Your study is exempt from further TWU Human Subjects Review because all of the subjects in your study are from Texas Christian University (TCU) and TCU's IRB has reviewed and approved this study.

___ No special provisions apply.

Sincerely,

John Engelbert

Chair
Human Subjects Review Committee

cc: Graduate School
Dr. Betty Alford, Nutrition and Food Sciences
Dr. Dorice Czajka-Narins, Nutrition and Food Science

TEXAS WOMAN'S
UNIVERSITY
DENTON DALLAS HOUSTON

THE GRADUATE SCHOOL
P.O. Box 425649
Denton, TX 76204-3649
Phone: 817/898-3400
Fax: 817/898-3412

January 22, 1996

Ms. Kimberly Gramenz
1340 Overlook Dr.
Lewisville, TX 75067

Dear Ms. Gramenz:

I have received and approved the Prospectus entitled "The Effects of an Acute Bout of Strenuous Aerobic Exercise on Plasma, Erythrocyte, Urinary, and Dietary Values for Selected Trace Minerals" for your thesis research project. Best wishes to you in the research and writing of your project.

Sincerely yours,

Leslie M. Thompson

Leslie M. Thompson
Associate Vice President for
Research and Dean of the
Graduate School

dl

cc Dr. Betty Alford